

Environmental Security Technology Certification Program (ESTCP)

FINAL REPORT

Portable SERS Instrument for Explosives Monitoring **Project CU-9917**



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LIST OF ACRONYMS

ALAAP	Alabama Army Ammunition Plant
ARA	Applied Research Associates
CCD	Charge-Coupled Device
CPT	Cone Penetrometer
CRREL	Cold Regions Research Environmental Laboratory
2,4-DNT	2,4-Dinitrotoluene
2,6-DNT	2,6-Dinitrotoluene
DoD	Department of Defense
EPA	Environmental Protection Agency
GAC	Granular Activated Carbon
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
HPLC	High Performance Liquid Chromatography
OSHA	Occupational Safety and Health Administration
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
SERS	Surface-Enhanced Raman Spectroscopy
SARM	Standard Army Reference Material
SW	Solid Waste
TNB	2,4,6-Trinitrobenzene

TNT	2,4,6-Trinitrotoluene
UMCD	Umatilla Chemical Depot
USACE	United States Army Corps of Engineers
VAAP	Volunteer Army Ammunition Plant

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EXECUTIVE SUMMARY

In this project, a new method based on surface-enhanced Raman spectroscopy (SERS) has been developed, fielded, and validated for field analysis of explosives in water samples. The SERS technique offers enhance performance over colorimetry and other field screening techniques for groundwater well, direct push and process water monitoring. When compared to the reference laboratory HPLC Method 8330 and field screening methods, the SERS method has the potential to reduce the time, cost, and waste generated per analysis while providing discriminate quantification of multiple analytes, even those within a chemical class, in a single measurement.

This project entailed three demonstrations at two Army facilities – Alabama Army Ammunition Plant (ALAAP) and Umatilla Chemical Depot (UMCD). The ALAAP demonstration was aimed at establishing that a SERS instrument could be brought from the laboratory to the field and used to perform explosives analysis on samples collected from groundwater wells. The purpose for the two demonstrations at UMCD was to extend the applicability of the SERS method from conventional groundwater well monitoring to include expedited site characterization from a cone penetrometer (CPT), direct push platform and at-line remediation process monitoring. Specific performance objectives of the demonstrations and the actual performance demonstrated to meet those objectives are summarized in the following table:

Type of Performance Objective	Primary Performance Criteria	Expected Performance (Metric)	Actual Performance
Qualitative	1. Fieldability	Successful fielding of the instrument	Fielded 5 times without any problems
	2. Ease of use	Potential operator acceptance	Three new users found SERS facile
	3. Matrix effects	Results not significantly affected by water parameters	No statistical bias in SERS results
Quantitative	1. Analytical performance (MDL, range)	Low $\mu\text{g/L}$ to $>100 \mu\text{g/L}$	MDL 2.6 to $5.1 \mu\text{g/L}$ for 5 major explosives; linear range to $500 \mu\text{g/L}$
	2. Spectral Resolution	Three or more analytes	Resolved four - RDX, HMX, TNT, and TNB
	4. Speed	$<15 \text{ min/sample}$	7-12 min/sample
	5. Waste generated	$< 5 \text{ mL/sample}$	1-3 mL/sample

Comparative statistical analysis of the data obtained by SERS for five prevalent explosive contaminants (the four listed in the table plus 2,4-DNT) in split water samples were in good agreement with Method 8330 results for the splits, validating the performance of SERS as an alternative to HPLC and colorimetric methods. Statistical analysis supported the following conclusions:

- Field SERS is an analytically acceptable alternative to HPLC or colorimetry for the analysis of explosives in water

- The performance of SERS in the field is comparable to laboratory SERS
- There is no consistent difference in SERS performance for different individual explosive analytes vs. the reference Method 8330
- There is no significant bias in the SERS method (i.e., matrix effects) and precision is better than interlaboratory reference method results (but considerably poorer than the single laboratory HPLC method for all analytes)

The SERS method was demonstrated to meet our most important analytical performance objectives by quantifying nitroaromatic explosives at low $\mu\text{g/L}$ concentrations linearly up to about 500 $\mu\text{g/L}$ without preconcentration; nitramines required a 10-fold preconcentration step to achieve comparable detection limits. The known effects of turbidity and extreme pH are controlled by filtration and pH adjustment, if required.

The SERS method did not demonstrate as high an accuracy and precision as the single laboratory reference Method 8330; however, it is not intended to be a routine replacement for laboratory HPLC. SERS does offer accuracy and precision comparable to multilaboratory HPLC and better than colorimetry. Considering that sampling error is generally far greater than analytical error, the capability of the SERS method should be sufficient to be accepted as an alternative to laboratory HPLC (and colorimetry) in most explosives monitoring scenarios.

In the course of the study, SERS demonstrated improved discrimination of explosives compared to colorimetric methods. At ALAAP, it was demonstrated that SERS could discriminate and quantitate TNT and 2,4-DNT in a single analysis whereas colorimetry could not quantitatively discriminate the two nitroaromatics. At the UMCD GAC Plant, four explosives were quantified simultaneously by SERS, whereas colorimetry could only discriminate the nitramine explosive class from the nitroaromatic class. Additionally, at ALAAP, three samples contained dyes that precluded colorimetric quantification (the sample absorbances actually decreased from their baseline levels when the colorimetric test for nitroaromatics was performed) but were successfully analyzed by SERS. In a second specific case, four groundwater samples collected with the CPT at UMCD tested falsely positive in the field for nitramines at about 2 $\mu\text{g/L}$ due to nitrate interference. Nitrate did not produce a false positive interference with the SERS method.

SERS offers considerable cost benefit to the user. The basic SERS method costs less than half the cost for a single colorimetric test. However, if both nitramines and nitroaromatics are tested (at a total cost of about \$80), basic SERS costs (no preconcentration) drop to just 25% of colorimetry and 15% of HPLC costs. With preconcentration, SERS costs are still less than half the expense of colorimetry for two tests and about 25% of HPLC costs. A summary of per-sample costs (excluding capital equipment) and capital equipment is presented in the table that follows:

SERS			Colorimetry		HPLC
Consumables	No Prec.	Preconc.	Consumables		
Colloidal Gold	\$1.00	\$3.00	Test Kit (SDI)	\$25.00	
Filter	\$1.00	\$1.00	Alumina Cartridge	\$2.00	
Vial, pipettes, etc.	\$2.00	\$3.00			
Solid Phase Cartridge	n/a	\$6.00			
Standards	\$1.00	\$1.00			\$150.00
Subtotal	\$5.00	\$14.00	Subtotal	\$27.00	
Labor			Labor		
Technician (\$60/hr)	\$15.00	\$20.00	Technician (\$60/hr)	\$15.00	
Total	\$20.00	\$34.00	Total	\$42.00	
Equipment			Equipment		>> 25 mL
Raman Spectrometer	\$52,000	\$52,000	Spectrophotometer	\$2,000	
Waste generated	1 mL	3 mL	Waste generated	25 mL	

SERS life cycle costs are dominated by capital equipment expenses that can be reasonably amortized over a five-year period. A useful means to compare the costs of different methods that include capital equipment is to calculate the break-even point. For SERS, the break-even point (i.e., the number of samples that must be analyzed to pay off the equipment and immediately realize the lower per-sample costs identified in the table) against Method 8330 is 400 samples without preconcentration and 450 samples with preconcentration. Assuming both nitramines and nitroaromatics are analyzed in each sample, the break-even point against colorimetry is about 850 samples without preconcentration and about 1100 samples with preconcentration – both are small numbers. Viewed from a different perspective, the break-even point can be reached with about \$90K of work, which is less than UMCD spends to monitor their GAC remediation process stream per annum.

In summary, this demonstration has proven some of the most important advantages of SERS, namely:

- Reliable quantification of important individual explosives in water samples at concentrations of regulatory relevance
- Faster results and lower cost than laboratory Method 8330
- Comparable speed, lower cost, simpler procedures, less matrix interference, and better discrimination of individual explosives compared to colorimetry
- Applicability to virtually any environmental water monitoring application such as groundwater well monitoring, expedited site characterization (CPT), and remediation process monitoring

1. Introduction

1.1 Background

The costs for initial characterization, remediation monitoring, and long-term, post-remediation monitoring of groundwater contaminated with explosives are increasing as live firing ranges, ammunition depots, ordnance test facilities and other DoD sites come under ever closer scrutiny. Major elements of the expense for characterization and monitoring are collection, packaging, shipping, and laboratory analysis of samples. The reference laboratory procedure for explosives is EPA SW-846 Method 8330, a high performance liquid chromatography (HPLC) method for analyzing 14 explosives and co-contaminants. Method 8330 is generally suitable for the analysis of water samples collected from groundwater wells. However, as pointed out by Jenkins and his colleagues¹⁻³ most samples test blank, wasting time and monetary resources on the laboratory procedure. Those samples that do test positive can be characterized by analyzing for just a few explosives, most notably TNT, 2,4-DNT and RDX, obviating the need for a complete Method 8330 analysis. Furthermore, the conventional approach of sampling and laboratory analysis is not well suited for monitoring active remediation processes such as "pump-and-treat" systems because turn-around times for laboratory results are too slow for process control.

An alternative to the current methodology is to use faster, less expensive, and more portable methods to perform measurements on water samples collected in the field. Indeed, field methods based on colorimetric and immunoassay techniques have been developed²⁻⁸ and have been used to screen groundwater collected from wells at sites such as Volunteer Army Ammunition Plant (VAAP) and for at-line monitoring of groundwater remediation processes at sites such as Umatilla Chemical Depot (UMCD). There are, however, limitations to both techniques such that the development and implementation of new approaches is warranted. For example, the time required for colorimetric analysis is quite long (approaching one hour) and a preconcentration step is required. Even the immunoassay time of 15 minutes per sample is not ideal for process control, especially when multipoint monitoring is desired. Although the costs for both methods are lower than for a laboratory Method 8330 HPLC analysis, faster methods offer to reduce costs even further.

A major limitation of both colorimetric and immunoassay methods is the range of applicability. The best immunoassay kits detect a single analyte and are available only for TNT and RDX. This limits their overall applicability to sites with these explosives and precludes their use for monitoring manufacturing impurities and the breakdown products of many remediation technologies. For example, at former ammunition manufacturing plants co-contaminants such as 2,4-DNT and 2,6-DNT are as important to monitor as TNT. The colorimetric methods have broader applicability than immunoassay techniques, with each colorimetric procedure responding to a class of chemicals such as nitroaromatics or nitramines. Repeating tests under different conditions (e.g., higher pH) can be used to provide some limited selectivity within a class. While this makes colorimetry more generally applicable at explosive sites, it also limits the ability to quantitate specific analytes when multiple compounds in the same chemical class are present in a sample. At VAAP and Alabama Army Ammunition Plant (ALAAP), where significant

quantities of 2,4-DNT and 2,6-DNT have been found to be present individually and as co-contaminants with TNT, use of the colorimetric procedure for nitroaromatics has proven to be of limited utility because responses to all three compounds cannot be resolved.⁹ With the colorimetric method the potential for chemical and spectral interference is also higher than for immunoassay, although sample matrix effects and cross-reactivity of the immunoassay technique can be significant and vary nonlinearly with concentration. In a comparison of eight methods conducted at several sites contaminated with explosives, it was observed that the accuracy of the techniques depended on site-specific groundwater quality parameters and concluded that no single field analytical method consistently outperformed the other methods.⁸ The analytical techniques used in the comparison included only colorimetry and immunoassay. During the study and follow-on work at UMCD, the colorimetric methods have proven most accurate and robust for routine groundwater and remediation process monitoring at the plant.^{8, 10}

In this project, a new method based on surface-enhanced Raman spectroscopy (SERS) has been developed, fielded, and validated for field analysis of explosives in water samples. The SERS technique offers enhance performance over colorimetry and other field screening techniques for groundwater well, direct push and process water monitoring. When compared to the reference laboratory Method 8330 and field screening methods, the SERS method has the potential to reduce the time, cost, and waste generated per analysis while providing discriminate quantification of multiple analytes, even those within a chemical class, in a single measurement.

1.2 Objectives of the Demonstrations

This project entailed three demonstrations at two Army facilities – ALAAP and UMCD. The ALAAP demonstration was aimed at establishing that a SERS instrument could be brought from the laboratory to the field and used to perform explosives analysis on samples collected from groundwater wells. The purpose for the two demonstrations at UMCD was to extend the applicability of the SERS method from conventional groundwater well monitoring to include expedited site characterization from a cone penetrometer (CPT), direct push platform and at-line remediation process monitoring. Specific objectives of the demonstrations were the following:

- (1) Demonstrate the general fieldability and ease of use of the SERS instrument.
- (2) Demonstrate capability for quantifying multiple explosives (TNT, 2,4-DNT, TNB, RDX and HMX) in a single measurement.
- (3) Demonstrate capability for at-line remediation process monitoring using the SERS method.
- (4) Demonstrate capability for *in situ* and *ex situ* groundwater monitoring from a CPT platform.
- (5) Demonstrate improved capability for discriminating explosives vs. colorimetry.
- (6) Demonstrate the cost benefit of the SERS technology.

The first demonstration was conducted at ALAAP in conjunction with regular well sampling being performed by trained SAIC personnel under contract to perform this service as part of the site's groundwater monitoring program. A total of 24 groundwater wells were sampled; four of

the wells were sampled a second time at two depths, giving a total of 32 groundwater samples from the site. The samples were split and subjected to field, laboratory, and reference laboratory analyses as described later in the Experimental Design section of this report. Of the 32 groundwater samples, 11 did not contain detectable quantities of explosives. The remaining 21 samples included two samples with only TNT, 11 samples with only 2,4-DNT, and eight samples with both analytes. Other explosives were not detected in the samples.

The second and third demonstrations were conducted in the area of the Explosives Washout Lagoons at UMCD in Hermiston, OR. This area has an RDX-led, mixed explosives groundwater plume that has undergone limited characterization. The known source location has been under remediation by a pump-and-treat (GAC) system since 1997. The extent of the explosives plume has not been well characterized by the limited well drilling program implemented to date. Thus, the second demonstration focused on using SERS to delineate the Eastern boundary of the contaminant plume to assist with placing a sentinel well. Site geology proved difficult to penetrate with conventional CPT, so an enhanced CPT technology that incorporated air rotary drilling was used to reach groundwater (ca 100 ft bgs) at 6 locations. As with the ALAAP samples, the UMCD groundwater samples were split for field and laboratory analysis. RDX was detected in two of the samples. In addition, SERS analysis was performed in the CPT probe but the low concentrations of RDX precluded *in situ* detection.

The third demonstration was performed at the UMCD GAC plant where at-line analysis was performed at the influent, two intermediate, and effluent points in the process in conjunction with routine sampling being performed by trained SCS Engineers personnel under contract to perform this service. A series of three sampling “events” over a period of several months produced a total of 12 discrete process samples for split field and laboratory analysis. The influent samples contained four explosive analytes (TNT, TNB, RDX, and HMX) whereas the intermediate samples contained low concentrations of RDX only and all three effluents were clean.

As will be described in the remainder of this report, all six of the cost and performance objectives of this project have been met. More specifically, the first objective (fieldability/ease of use) was achieved on five occasions when the maintenance-free SERS instrument was successfully fielded, setup and checked out in less than an hour, and operated for the duration of the demonstration without a single difficulty. SERS analyses require a minimal amount of sample preparation; three technicians without previous Raman experience were trained in the SERS method in a couple of hours. All three technicians found SERS simpler to perform than the colorimetric methods, with which they had previous experience. A complete SERS analysis was demonstrated to be performed in 7-12 minutes, generating only 1-3 mL of waste which is negligible compared to other methods and within our goal of 5 mL/sample.

The second goal, quantification of multiple analytes in a single analysis, was best demonstrated at the UMCD GAC Plant where four explosives – TNT, TNB, RDX, and HMX were measured simultaneously in the process influent stream. As discussed in the statistical analysis section, the analytical results obtained with SERS for all analytes (the four above plus 2,4-DNT) were in good agreement with Method 8330 results, validating the performance of SERS as an alternative

to HPLC and colorimetric methods. The SERS method was demonstrated to meet our most important analytical performance objectives by quantifying nitroaromatic explosives at low $\mu\text{g/L}$ concentrations linearly up to about 500 $\mu\text{g/L}$ without preconcentration; nitramines required a 10-fold preconcentration step to achieve comparable detection limits. Furthermore, there was no statistically significant bias in the SERS results, demonstrating that the method was not subject to water matrix effects. The known effects of turbidity and extreme pH are controlled by filtration and pH adjustment, if required. There was no requirement for pH adjustment for the samples analyzed in this study. The SERS method did not demonstrate as high an accuracy and precision as the single laboratory reference Method 8330; nevertheless, it was not intended to be a routine replacement for laboratory HPLC. SERS does offer accuracy and precision comparable to multilaboratory HPLC and better than colorimetry. Considering that sampling error is generally far greater than analytical error,¹ the capability of the SERS method should be sufficient to be accepted as a replacement for HPLC (and colorimetry) in nearly all explosives monitoring scenarios.

The third objective, process monitoring, was met with three successful fieldings of the SERS instrument at the UMCD GAC groundwater treatment plant. SERS process monitoring performance was again validated through statistical agreement with Method 8330 and colorimetry (the method currently being used for process monitoring) results.

In situ and *ex situ* CPT SERS were also demonstrated at UMCD. Although the *in situ* equipment worked as designed and successfully analyzed a spiked groundwater sample up-hole, analyte concentrations were below *in situ* detection limits down-hole (sample preconcentration could not be used *in situ*). Therefore, analytical *in situ* performance could not be validated through a comparison of methods. However, the analytical performance of *ex situ* SERS, which is expected to be used with the CPT far more often than *in situ* SERS (because of better sensitivity, simpler implementation, and lower cost), was validated through statistical agreement with the HPLC laboratory method and colorimetry.

The fifth objective, improved discrimination of explosives against the colorimetric methods, was demonstrated generally and several times specifically, despite the fact that most of the samples encountered in this study were “colorimetry friendly” (i.e., not turbid or highly colored with humic materials – common nemeses of colorimetry). At ALAAP, it was demonstrated that SERS could discriminate and quantitate TNT and 2,4-DNT in a single analysis whereas colorimetry could not quantitatively discriminate the two nitroaromatics (qualitatively, blue samples are predominantly 2,4-DNT and red samples contain primarily TNT). At the UMCD GAC Plant, four explosives were quantified simultaneously by SERS (with good agreement with Method 8330), whereas colorimetry could only discriminate the nitramine explosive class from the nitroaromatic class. Additionally, at ALAAP, three samples contained dyes that precluded colorimetric quantification (the sample absorbances actually decreased from their baseline levels when the colorimetric test for nitroaromatics was performed) but were successfully analyzed by SERS (and Method 8330). In a second specific case, four groundwater samples collected with the CPT at UMCD tested falsely positive in the field for nitramines at about 2 $\mu\text{g/L}$ due to nitrate

interference (an alumina nitrate removal cartridge was not available at the time). Nitrate did not produce a false positive interference with the SERS method (or Method 8330).

The cost benefit of the SERS technology is discussed in detail in Section 5 of this report. Tracking costs in this project, we were able to determine the break-even point for SERS against Method 8330 and colorimetry, after which the SERS costs are less than 25% and 50% of the other methods, respectively. Under worst-case scenarios, the break-even point against Method 8330 is approximately 450 samples and 1100 samples against colorimetry – both are small numbers. Viewed from a different perspective, the break-even point can be reached with about \$90K of work (a single major project), which is less than UMCD spends to monitor the GAC plant per annum. There is little question that cost benefit can be realized with SERS in a year or less.

In summary, this demonstration has proven some of the most important advantages of SERS, namely:

- Reliable quantification of important individual explosives in water samples at concentrations of regulatory relevance
- Faster results and lower cost than laboratory Method 8330
- Comparable speed, lower cost, simpler procedures, less matrix interference, and better discrimination of individual explosives compared to colorimetry
- Applicability to virtually any environmental water monitoring application such as groundwater well monitoring, expedited site characterization (CPT), and remediation process monitoring

1.3 Regulatory Drivers

The EPA has not established health standards for explosives such as TNT and RDX in water, however health advisories have been issued.¹¹ At virtually all DoD sites where groundwater has been found to be contaminated with explosives at $\mu\text{g/L}$ to mg/L concentrations, regulators require groundwater well sampling and analysis as a major component of clean-up programs. During the remediation and post-remediation phases of clean up, which can last up to decades, monitoring is required for process control, performance measurement, and compliance. The extended duration and expense of required monitoring programs create the need for faster, better performing, and lower cost monitoring technologies such as SERS, as proven in this demonstration project (see Section 1.2).

1.4 Stakeholder/End-User Issues

At ALAAP, immunoassay and colorimetric field methods have in the past been used to help contain groundwater well and surface water monitoring costs. However, the practice was discontinued because of cross reactivity, background interference and the inability to quantify individual nitroaromatic species in samples containing multiple analytes. U. S. Army Corps of Engineers (USACE) - Mobile District personnel with oversight responsibilities and the

contractor (SAIC) performing analytical work at the site have expressed interest in using new methodologies if they are sensitive to the low $\mu\text{g/L}$ concentration range and can discriminate between TNT, 2,4-DNT and 2,6-DNT. Following the success of the demonstration, SAIC is considering the purchase of a Raman (SERS) instrument for use in their explosives groundwater monitoring effort.

At UMCD, colorimetric field methods have been used for years to help speed up and contain monitoring costs at the pump-and-treat remediation facility. However, the practice has at times been complicated by cross reactivity, background interference and the inability to quantify individual species in samples containing multiple analytes. USACE - Seattle District personnel with oversight responsibilities and the contractor (SCS Engineers) performing analytical work at the plant have expressed interest in using new methodologies if they are sensitive to the $\mu\text{g/L}$ concentration range and can discriminate between TNT, RDX and other major explosives that may be present at some times (e.g., TNB and HMX). Following the success of the demonstration effort, SCS Engineers and the USACE are seeking to fund further development of the SERS technology for remote, unattended, multipoint process monitoring.

The extent of the explosives plume at the UMCD lagoons is not well understood due to the limited number of monitoring wells drilled at the site. There is a strong desire among Army Corps engineers to better delineate the plume. The CPT demonstration showed that with an enhanced CPT system and SERS, the explosives groundwater plume can be rapidly and cost-effectively delineated. A follow-on CPT SERS program for more extensive plume characterization and placement of sentinel wells is planned for the UMCD lagoons.

This project has lead to the development of a reliable, rapid, and cost-effective method for explosives screening and compliance monitoring. The SERS method is ready for implementation at DoD installations and other sites. EPA's recent acceptance of performance-based standards should allow many sites to deploy the technology, using the results of this demonstration to support the use of the method.

2. Technology Description

2.1 Technology Development and Application

Raman spectroscopy is a high-resolution, vibrational spectroscopic technique where each molecule produces a unique spectral “fingerprint” that can be used to identify and differentiate it from other sample components (see Figure 4 later in this section for an example). Recent advances in lasers, detectors, and optical filter technologies have enabled considerable downsizing of Raman instrumentation such that field deployment is now feasible.

For this project, we assembled the portable Raman system depicted in Figure 1 to perform SERS analysis. The major instrument components are a wavelength stabilized diode laser (785 nm) and compact spectrograph equipped with an air-cooled multichannel CCD detector interfaced to a portable computer for data collection and processing. Both the laser and spectrograph are of

shoebox size and are coupled to a duplex fiber optic Raman probe as depicted in Figure 1 and photographed in Figure 2. One optical fiber guides laser light to the sample; the second optical fiber delivers backscattered Raman light to the spectrograph for detection. For *ex situ* ground or process water monitoring such as was performed with groundwater samples collected at ALAAP, the fiber optic probe is relatively short (3 m fiber length) and terminates in a sampling chamber into which water samples are introduced in small cuvettes. The sample chamber holds the Raman probe in proper alignment to the sample and blocks ambient light from reaching the probe during the measurement. A sliding cover allows samples to be quickly swapped in and out of the chamber.

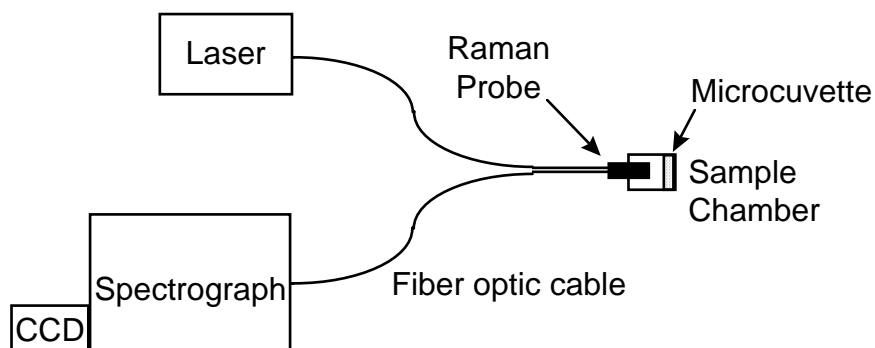


Figure 1. Schematic representation of the portable Raman instrument.



Figure 2. Photograph of the portable Raman instrument in the trunk of a rental car at ALAAP. The laser (blue) is sitting on top of the spectrograph (white) with a multichannel CCD detector (light blue) attached. In the foreground is the fiber optic Raman probe inserted in the sample chamber. Reagents for the SERS method are in the muffin tin.

Conventional Raman scattering is not a strong phenomenon, with lower detection limits in the high mg/L range for the strongest Raman scatterers in water. Fortuitously, water is an extremely weak Raman scatterer exhibiting just a few broad spectral bands outside the fingerprint region such that it does not interfere with most Raman analyses. SERS is a variation of conventional Raman spectroscopy whereby analytes are adsorbed onto a noble metal surface prior to analysis. Through a combination of chemical and electromagnetic effects, the Raman signal intensity is “enhanced” by as much as 10^6 in SERS. Again, water exhibits little or no surface enhancement effect and does not interfere. Although a diversity of metal surfaces has been successfully implemented for SERS, we have developed a simple "cocktail" using aggregated, commercially available colloidal gold particles for Raman enhancement. Sample preparation is straightforward, involving just a mixing of 0.5 mL of colloidal gold formulation with 0.5 mL filtered water sample, waiting 1 min, and then performing Raman analysis for 5 min. The colloid formulation is buffered to ensure that aggregation of the gold particles is consistent and the responses are therefore reproducible. Greater sensitivity can be achieved by preconcentrating explosives on solid phase extraction media using standard procedures developed for colorimetric water assays.^{2,8}

As part of our SERS method development effort we determined that the two major sample factors affecting performance were pH and turbidity. High turbidity reduces SERS response by blocking the laser beam entering the sample cuvette. Deleterious turbidity effects are avoided by filtering samples through 0.45 μm syringe-type water filters as is the standard practice with other field and laboratory methods.^{1-3,7,8} The effect of sample pH on SERS response is shown in Figure 3. SERS response is reduced at pH extremes that exceed the buffering capacity of the colloidal gold formulation. At low pH (<3) there is insufficient aggregation of the colloidal gold particles to provide a strongly enhancing surface whereas at high pH (>12) there is excessive particle aggregation and formation of precipitates. Optimum SERS response was found at about pH 10.5 which led us to buffer our colloidal gold formulation at that pH.

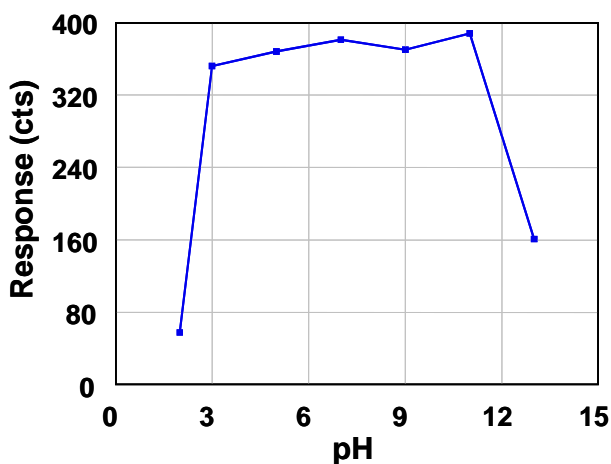


Figure 3. Effect of sample pH on SERS response to 100 $\mu\text{g/L}$ TNT in water.

Another element in the project was development of a deconvolution method for quantifying multiple analytes in a sample. An example of the power of the method is shown in Figure 4 where a minor quantity of 2,6-DNT was resolved from larger quantities of TNT and 2,4-DNT.

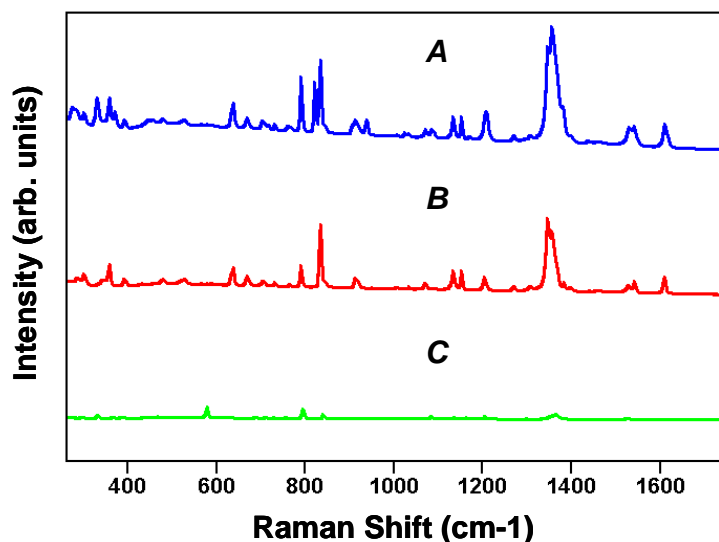


Figure 4. SERS spectral deconvolution enables isolation and quantification of individual analytes in a mixture. (A) Mixture of TNT, 2,4-DNT, and 2,6-DNT; (B) TNT spectrum removed; (C) Both TNT and 2,4-DNT spectra removed leaving just the 2,6-DNT spectrum.

As accessories to the standard Raman (SERS) equipment described above, sampling units have been devised for special applications such as process monitoring or *in situ* CPT measurements. These samplers incorporate the standard fiber optic Raman probe as the essential measurement element. For process monitoring at low measurement frequency, grab sampling and immediate analysis is an acceptable approach to reduce costs. However, if frequent measurements are desired or if getting personnel to the site is difficult, then an automated fluid handling and analysis system can further reduce costs by minimizing operator time. Although manufacture of a dedicated, unattended automated analyzer was beyond the scope of this project, we developed a process interface that meets the basic fluid handling needs of an unattended SERS analyzer. A general depiction of the fluidics handler is presented in Figure 5. Filtered water sample is delivered into the fluidics system via water pressure (process) or pneumatic pressure (CPT). A switching valve (solid circle) directs sample into the SERS measurement cell or to a “grab” sample container located external to the fluidics unit. When SERS measurement is desired, the reagent pump is turned on for a few seconds and reagent is mixed with sample into the measurement cell. Flow is then stopped (both valves are closed) during the measurement period. Grab sample can be collected during the SERS measurement.

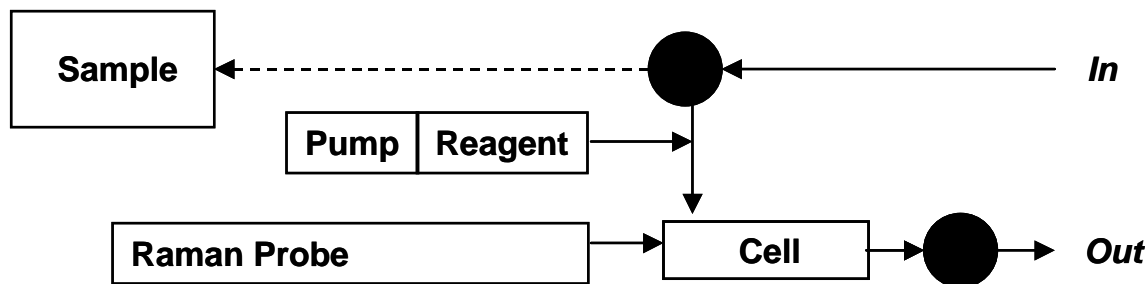


Figure 5. General schematic of fluidics handling system for *in situ* SERS analyzers.

Figure 6 is a photograph of the cylindrical fluidics unit with the Raman probe and electrical cable installed in it. The unit can be interfaced to a process line or inserted into a CPT probe or rod to perform *in situ* SERS measurements and collect samples down-hole. We installed and tested the unit in our ConeSipper™ CPT water sampler and behind our Wireline CPT water sampler, described below. However, as noted earlier, these direct push tools were not applicable at UMCD (which required drilling – direct push was not feasible) and we instead configured the fluidics unit above a commercial bladder pump sampler for *in situ* detection.



Figure 6. Fluidics unit with Raman probe and electrical cable.

At most site characterization projects using CPT, the preferred operational mode will be to collect a groundwater sample and then perform a rapid analysis up-hole, rather than *in situ* (down-hole). This approach eliminates the need for putting expensive components such as the Raman probe down-hole and can provide much larger volumes of sample when preconcentration or multiple analyses are required (e.g., split confirmatory samples). We have developed an innovative water sampler for our Wireline CPT system. ARA's Wireline CPT allows for the exchange of down-hole tools and sensors without removal of the rod string; the down-hole components are simply unlocked and retrieved from the rod string using a steel cable.¹² The Wireline water sampler is shown in Figure 7. The heart of the device is a pneumatically driven, miniature bladder pump that delivers groundwater to the surface from depths as great as 200 ft below ground surface (bgs).

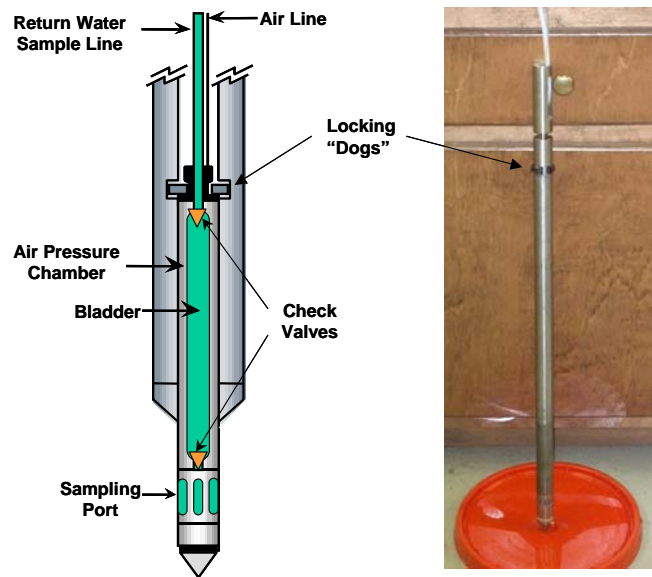


Figure 7. Wireline CPT water sampler.

There are many sites, including UMCD, where conventional CPT meets refusal before reaching groundwater. For these cases, ARA has developed an enhanced CPT system that combines overburden drilling with conventional CPT. When necessary, air-rotary CPT drilling is used to penetrate consolidated layers impervious to conventional CPT. A photograph of the integrated CPT/drill head in ARA's enhanced CPT rig is presented in Figure 8. If drilling is required in the groundwater, then the Wireline water sampler is not used, but rather a commercial bladder pump can be deployed after removing the center drill bit and rod string from the outer casing. A photograph of the commercial (Durham Geo) bladder pump with the fluid-handling unit configured above the pump is shown in Figure 9.

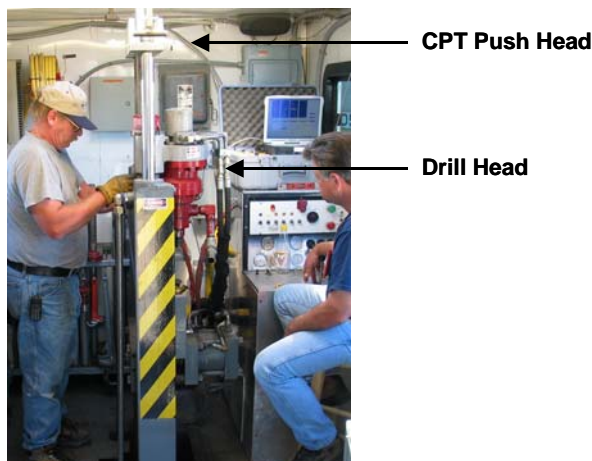


Figure 8. Enhanced CPT system with integrated rotary air drilling capability.



Figure 9. Commercial bladder pump with fluid handling unit configured above it.

The SERS method in its various embodiments can be applied widely at explosives-contaminated sites including manufacturing plants (both military and commercial), firing ranges, etc. The water analysis method can be used at all stages of cleanup (site characterization through post-remediation monitoring) and, with modification, could also be used to field screen soil samples. In this project, we demonstrated that the method could be employed for groundwater well monitoring, remediation process monitoring, and CPT-based groundwater plume characterization.

2.2 Previous Testing of the Technology

Prior to this project, the principal investigator^{13,14} and others^{15,16} had demonstrated in the laboratory that SERS could be used to sensitively detect and identify a few explosives-related compounds such as TNT in water or air matrices. However, the methods were not optimized, investigated with mixtures of analytes or real-world samples, nor evaluated for analytical performance in the laboratory or in the field. All of these advancements were achieved in the current project as described in this report.

2.3 Factors Affecting Cost and Performance

For comparison with other technologies, the cost of the SERS technology is primarily driven by the following factors:

- (1) Cost of the SERS instrument amortized over its useful life.
- (2) Reagent costs per sample.
- (3) Labor cost per analysis (including operator training costs).
- (4) Waste disposal costs per sample (determined to be negligible)

When standard water sample collection procedures are used, as in the first demonstration at ALAAP, it can be assumed that sample collection costs are approximately the same for all field

and laboratory methods, including SERS. It is also assumed that reporting costs are comparable for all methods. Therefore, field methods such as SERS and colorimetry save the costs of sample transport and storage and also provide more timely results to decision makers when compared against laboratory methods. When measurements are performed unattended (e.g., in process monitoring), the costs for sample collection are also eliminated, as are the labor costs associated with the analysis.

In the special case of CPT SERS, the general comparative cost analysis for SERS vs. other methods is still valid (i.e., the CPT can be viewed as a sample collection tool). Indeed, the predominant cost for CPT SERS is the CPT operations, not the analysis. Thus cost comparisons for CPT SERS are best made against conventional drilling and sampling. The savings of CPT over drilling are already well documented.¹⁷⁻¹⁹

As discussed in Section 2.1, the two major factors affecting SERS performance are sample pH and turbidity. The optimum pH for SERS detection of explosives is 10.5. The pH is regulated (buffered) in the colloidal gold "cocktail" mixed with the sample; however, samples outside the pH range 3-12 produce low results. Adjustment of the pH with acid or base prior to adding colloidal gold easily resolves this problem. However, the occurrence of environmental water samples at pH extremes is rare. In this project all water samples tested neutral (pH 6.5-7.5) and pH adjustment was never required.

Turbidity is a problem for nearly all optical methods (due to attenuation of incident light) and other methods as well. All of the methods used for analyzing explosives in water samples (SERS, colorimetry and the HPLC reference method in this project) are adversely affected by turbidity. Analytical SERS results are biased low and colorimetric results are biased high in turbid samples. Turbid samples plug HPLC tubing and columns. Therefore, filtering is recommended in EPA-approved colorimetric (Method 8515) and HPLC (Method 8330) sample preparation protocols and we routinely employed it with the SERS method in this project. All of our water sampling systems incorporated filters at the inlets. In general, filtering is a well-accepted practice for water samples being analyzed for nonvolatile organic compounds and should meet with no objections from regulators.

2.4 Advantages and Limitations of the Technology

The principal advantages of SERS over the reference HPLC analytical Method 8330 are speed, cost, and waste generated. By eliminating sample packaging, shipping to a contract laboratory, storage, preparation and a lengthy laboratory analytical procedure the field SERS method can significantly reduce the cost per sample and reduce the turn-around time for reporting results from days or weeks to about 10 min. A further advantage of SERS is that samples of about 1 mL volume are analyzed. The result is very little waste generated by the method, saving on expensive disposal costs. This efficiency contrasts markedly with the reference HPLC method, which generates 100-fold or more waste volume per sample compared to the SERS method. The SERS method also generates less waste than the colorimetric and immunoassay methods,

although the reductions are not as significant (near 10-fold). Working with small samples also saves on reagent costs.

Analytical performance, described in detail in Section 4, is the primary limitation of SERS vs. Method 8330. Without sample preconcentration, SERS detection limits are higher than the HPLC method, especially for the nitramines. As measured by percent relative standard deviation, the precision of Method 8330 is also nearly an order of magnitude better than SERS. The accuracy of Method 8330 is slightly better than SERS. However, as noted previously, sampling error is normally much larger than analytical error, so the performance advantages of the reference method may not be realized.

A major advantage of SERS over colorimetric and immunoassay field screening procedures is selectivity. Raman spectroscopy produces a unique spectral "fingerprint" for every molecule, as shown in Figure 10 for TNT, 2,4-DNT, and 2,6-DNT. The three compounds have closely related chemical structures with identical sets of chemical functionalities (i.e., nitro, methyl, C-N, etc.), yet they are easily distinguished by the Raman technique (see the starred peaks in the figure). This capability for resolving and quantifying multiple species in a single analysis (after spectral deconvolution as discussed in Section 2.1) is especially useful in monitoring situations where mixtures of explosives and related compounds are present. An example is shown in Figure 11 for a UMCD groundwater sample that contains detectable quantities of four explosives (TNT, TNB, RDX, and HMX), all of which can be spectrally resolved and quantified. Colorimetric and immunoassay techniques cannot offer this advantage – each test responds to a single analyte or class of analytes (e.g., nitroaromatics or nitramines). Multiple tests are required to quantify individual analytes or classes of analytes in mixtures (within the ultimate capabilities of the method – e.g., colorimetry cannot resolve RDX from HMX) at additional time and monetary cost.

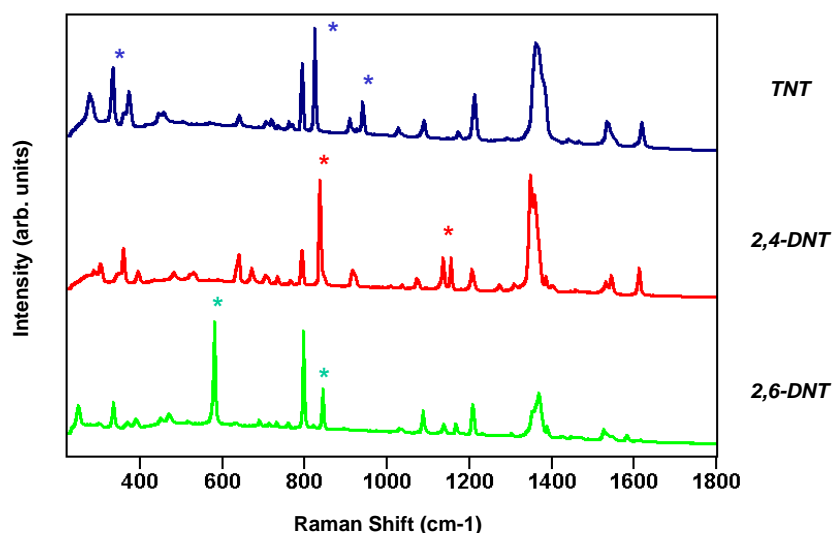


Figure 10. Raman spectra of nitroaromatic explosives. Strong, unique spectral features are highlighted with stars.

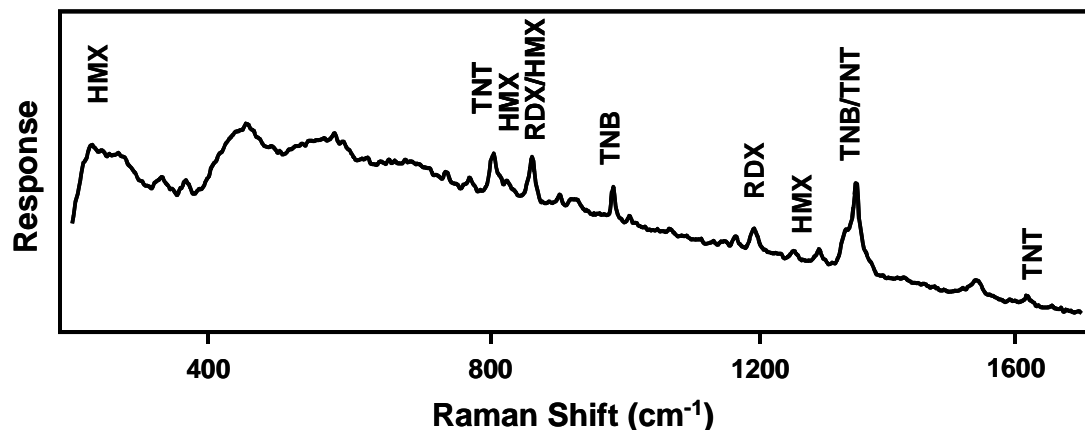


Figure 11. SERS spectrum of a mixture of RDX, HMX, TNT, and TNB in UMCD groundwater.

Other metrics of analytical performance (detection limits, accuracy, precision, etc.) are comparable or favor SERS over colorimetry and immunoassay. Depending on the specifics of a site, additional potential advantages of SERS over colorimetry and immunoassay methods include speed, cost, and reduced sample matrix effects. For sites with a single analyte and a “clean” water matrix the speed and cost of the field methods are similar.

3. Demonstration Design

3.1 Performance Objectives

The performance objectives for the SERS demonstrations are summarized in Table 1 and formed the basis for evaluating the cost and performance of the SERS technology. As shown in the table, all of the qualitative and quantitative objectives of the demonstrations have been met. Our cost goal was \$75 or less to quantify up to three analytes per sample, which is similar to single analyte immunoassay and colorimetry (undifferentiated analytes) costs and much less than the reference, two-column HPLC laboratory method (about \$250 for one analyte and \$25 for each additional analyte) with 30-day data turnaround. Laboratory costs vary widely for rapid turnaround samples, but can be as high as \$1,000.00 per sample.

Table 1. Performance Objectives

Type of Performance Objective	Primary Performance Criteria	Expected Performance (Metric)	Actual Performance
Qualitative	1. Fieldability	Successful fielding of the instrument	Fielded 5 times without any problems
	2. Ease of use	Potential operator acceptance	Three new users found SERS facile
	3. Matrix effects	Results not significantly affected by water parameters	No statistical bias in SERS results
Quantitative	2. Analytical performance (MDL, range)	Low $\mu\text{g/L}$ to $>100 \mu\text{g/L}$	MDL 2.6 to 5.1 $\mu\text{g/L}$ for 5 major explosives; linear range to 500 $\mu\text{g/L}$
	2. Spectral Resolution	Three or more analytes	Resolved four - RDX, HMX, TNT, and TNB
	4. Speed	$<15 \text{ min/sample}$	7-12 min/sample
	5. Waste generated	$< 5 \text{ mL/sample}$	1-3 mL/sample

3.2 Selecting Test Sites

ALAAP was selected as the first test site because it had: (1) significant quantities of multiple explosive analytes in the groundwater, (2) over three dozen groundwater wells, (3) difficulties with conventional field screening and Method 8330 analyses that indicated potential challenges to SERS and the other methods, (4) an ongoing groundwater monitoring program with a significant body of historical data, and (5) a receptive on-site contractor (SAIC). An alternate site, VAAP, had similar characteristics but had a discontinuous groundwater sampling program which was not active at the time of the demonstration.

The UMCD facility was selected for the second test site because it satisfied the need for two demonstration sites – a groundwater remediation process and a subsurface plume of contamination. More specifically, the Explosives Washout Lagoons at UMCD had: (1) a known history of mixed explosive analytes in the groundwater, and (2) an ongoing groundwater monitoring program with historical data and a current groundwater model. The groundwater remediation system is an actively operating pump-and-treat (GAC) system with sampling points in place. The UMCD facility was also readily accessible to ARA's Richland, WA-based CPT rig, which has enhanced access (drilling) capabilities. The USACE in Seattle, WA also maintained considerable interest and provided valuable infrastructural support to the UMCD demonstrations.

3.3 Test Site Descriptions

ALAAP is located near Childersburg, AL. Smokeless powder, nitrocellulose and nitroaromatic explosives were manufactured at the site until 1988 when the plant was officially closed. Military activity is nonexistent. Originally over 13,000 acres in size, much of the site has been sold to private concerns with about 2200 acres remaining under USACE – Mobile District -

oversight. ALAAP is unoccupied and a single small building that supports the on-going groundwater and surface water monitoring programs remains on the site. Historically, the primary groundwater contaminants identified at the site have been TNT, 2,4-DNT, and 2,6-DNT although 2,6-DNT was not detected during the demonstration. Previous manufacturing practices suggest that tetryl could also be found in groundwater, but has not yet been detected. Groundwater pH is neutral and generally flows in the direction of Talladega Creek, which is fortunately away from local municipal drinking water wells. From about 2000-2002 a series of over two dozen wells were developed at ALAAP. Additional wells have recently been constructed at locations near and outside the perimeter of the Alabama plant. Water sampling and analysis has not followed a regular quarterly schedule at the site but has been conducted at least twice a year. The schedule is impacted by local rainfall conditions, because analyte concentrations have typically been highest following periods of heavy rainfall. Thus, spring and fall sampling is normally conducted as soon as possible after heavy rainfall events so that conservative estimates of groundwater contamination can be made.

UMCD is located in northeastern Oregon in Morrow and Umatilla Counties, approximately 5 miles west of Hermiston, Oregon. The installation covers about 19,700 acres of land. UMCD was established as an Army ordnance depot in 1941 for the purpose of storing and handling munitions. From the 1950's until 1965 UMCD operated an explosives washout plant onsite. Munitions were opened and washed with hot water to remove and recover explosives. The plant was cleaned weekly, and the washwater was disposed in two nearby lagoons where it percolated into the soil. The lagoons received an estimated total of 85 million gallons of washwater during plant operations. Although lagoon sludges were removed regularly during operation, explosives contained in the washwater migrated into the soil and groundwater 47 ft beneath the lagoons. There is a pronounced west-to-east gradient in depth to groundwater at the site, reaching 100-120 ft on the eastern edge of the plume. Because of the soil and groundwater contamination (RDX, TNT, HMX, and TNB) the lagoons were placed on EPA's National Priorities List in 1987. The pH of groundwater in the lagoons has historically been neutral.

As the first step to remediation of the site, the contaminated soil in the lagoons was removed in September 1994 and subjected to bio-treatment. The pump-and-treat (GAC) remediation system was installed later to treat explosives contaminated groundwater and has been in operation since 15 January 1997. Based on pump-and-treat influent monitoring data and quarterly groundwater well monitoring. The RDX plume has been reduced slightly in size and concentrations have been reduced more than an order of magnitude in the center of the plume. However, in the northeast portion of the plume, concentrations have not declined during pump-and-treat operations. Therefore, the Army Corps desires to better characterize the magnitude and extent of contamination in that area with the additional objective of placing a series of sentinel wells outside the minimum zone of contamination (2.1 µg/L).

Pump-and-treat remediation of the groundwater in the UMCD Explosives Washout Lagoons continues in full-scale operation today with oversight by the USACE - Seattle District. Regular groundwater remedial action monitoring is an integral part of the ongoing remediation program. Monitoring is accomplished using conventional sampling and contract laboratory colorimetric

analysis with occasional (about annual) Method 8330 HPLC analysis. As discussed earlier in this report, the Army Corps has identified a need to better characterize the RDX plume in the northeast region of the site where concentrations have not been reduced by pump-and-treat operations. There is a potential concern that contaminants may be migrating in that direction, beyond the outermost monitoring wells. Thus, it is desirable to better define the plume boundary in that area and place sentinel wells to provide early detection of future contaminant migration.

3.4 Pre-Demonstration Testing and Analysis

Because our approach to SERS evaluation used comparative analysis of methods on split samples, extensive pre-demonstration testing and analysis to provide baseline data was not required. However, prior to the ALAAP demonstration we analyzed 10 groundwater well samples from the site by laboratory HPLC and SERS to ensure that no unexpected difficulties were encountered with the SERS method. The samples were collected from stagnant wells following a prolonged dry period at the site, and therefore did not contain high concentrations of explosives. Each sample was analyzed for TNT, 2,4-DNT, and 2,6-DNT by HPLC Method 8330 and the SERS method; the results are summarized in Table 2.

Table 2. Pre-Demonstration Results (µg/L) for ALAAP Groundwater Samples

Sample	TNT SERS	TNT HPLC	2,4-DNT SERS	2,4-DNT HPLC	2,6-DNT SERS	2,6-DNT HPLC
03-06	nd*	nd	3	2	nd	nd
03-07	5	2	nd	2	nd	10/nd
04-01	nd	nd	23	19	nd	nd
04-05	nd	nd	nd	nd	nd	50/nd
04-06	30	25	70	51	nd	nd
07-01	nd	nd	nd	nd	nd	nd
17-22	nd	nd	nd	nd	nd	nd
12-23	nd	nd	nd	nd	nd	nd
18-09	110	130	5	2	nd	nd
20-04	nd	nd	nd	nd	nd	nd

* nd = not detected

As expected, the levels of the explosives were generally low, with only half the samples containing detectable concentrations of nitroaromatics. Other samples had analytes near the low µg/L detection limits. Overall, there was good agreement of the SERS results with the HPLC results and therefore no indication that the samples posed any special difficulties for the SERS method. Using just a single C-18 HPLC column, samples 03-07 and 04-05 gave positive results for 2,6-DNT (10 and 50 µg/L, respectively). Subsequent analysis on a CN column indicated the C-18 results were false positives. Spectral analysis of the suspect peaks confirmed that the interferent was fluorescein dye.

At UMCD, there was five years of historical data from both the groundwater monitoring wells and GAC treatment system that could be used as a general guide for the concentrations that could be expected for the SERS technology. However, as in the ALAAP demonstration, we relied on a side-by-side comparison of methods (SERS, colorimetry, and Method 8330) using split samples in the field and laboratory rather than historical data to validate our method.

An important pre-demonstration uncertainty at UMCD was CPT penetrability. Therefore, we mobilized our enhanced CPT system to the site from Richland, WA in June 2003 and performed a series of CPT soundings. The results from three test locations in the northeast section of the Explosives Washout Lagoons clearly demonstrated that to reach groundwater the air-rotary drilling feature of the system was required. It was not possible to use conventional, quasi-static push CPT at depths below 11.3 ft on the site, which is well above the groundwater level (about 100 ft bgs). A short-duration, CPT rotary drilling test demonstrated that we could drill through the consolidated gravel layers at 11.3 ft and below. The uniformity of the gravel material reported in coarse drilling logs across the lagoon area suggested that conventional CPT would likely be precluded at the site, as was confirmed during the demonstration.

3.5 Testing and Evaluation Plan

A series of comprehensive demonstration plans was developed for the project and are described in the subsections below.

3.5.1 Demonstration Installation, Start-Up, and Fieldwork Period

Fieldwork at ALAAP was conducted the third week of May 2002 in conjunction with routine water sample collection at the site. Both the SERS and colorimetry equipment are portable and were shipped as airline baggage and transported to the site in a rental car. In about an hour, the equipment was unpacked and set up on a desktop in the sample processing building on site. A photograph of the instrumentation and associated sample preparation items (reagents, filters, pipettes, etc.) is presented in Figure 12. The building supplied 120 VAC power for the SERS computer, laser, and detector as well as the colorimeter. Chemical standards were used to confirm that the Raman spectrograph and colorimeter wavelength calibrations and detector responses had not changed (indicating potential equipment damage) during transport to the site.

SERS analyses were also performed in the back of the rental car at three well heads (see Figure 2). A 400 W inverter was used to power the SERS equipment from the car battery. Again, no changes in wavelength calibration or detector response were observed when driving the Raman instrument around the site. In general, performing measurements at wells is not efficient because sample collection takes far longer than the analysis. Instead, it is more cost effective to first collect the samples over a several day period and then perform the analyses in a single day (or less). We used this approach at ALAAP where about half of the samples were collected over a four-day period and preserved on ice prior to our arrival at the site (note the sample coolers in the background of Figure 12).



Figure 12. SERS (left) and colorimetry (right) methods set up on a desk at ALAAP.

In the course of the demonstration at ALAAP, the SERS and colorimetry instruments performed without any problems, maintenance, or need for re-calibration (as confirmed by daily calibration checks). There is no maintenance required for the Raman or colorimetry equipment. Sampling equipment and optical cells need to be cleaned between analyses. All other sample preparation items (e.g., pipettes, glass vials) are disposable. No safety issues were encountered during the demonstration.

At the three visits to the UMCD GAC plant (September 2003, November 2003, and January 2004) the SERS and colorimetry equipment was set up on a portable folding table in the same manner as at ALAAP (see Figure 12). AC power was available, so we did not need to use a car battery and inverter to power the computer and instruments. Again, daily instrument calibration and response checks revealed no changes (damage) to the equipment during cross country shipment (VT to WA), rental car transport to the site from Richland, WA or during the fieldwork periods. Each visit required a single day to mobilize to the site, set up, analyze the four process samples (influent, two intermediate, and effluent), and demobilize back to Richland, WA. The visits were coordinated with regular sampling being conducted by SCS Engineers.

For the two-week CPT demonstration at UMCD in mid-November 2003, the sampling and analytical equipment was installed in the back of the CPT truck, which also supplied 120 VAC power. As shown in Figure 13, there was ample room in the CPT truck for all of the equipment. About an hour was required to set-up the SERS and colorimetry apparatus and perform an initial calibration/response check. No deviations from expected performance were observed during the ensuing two-week demonstration period.



Figure 13. SERS (left) and colorimetry (right) apparatus installed in the CPT truck.

The ARA enhanced access CPT truck is operated out of Richland, WA. Therefore mobilization and set-up at the first UMCD penetration location required about half a day. Over the next two and a half days a series of rotary air drilling bits were investigated for optimal penetration of the UMCD subsurface as the first penetration to groundwater was conducted. This “shakedown” effort proved worthwhile as a drill bit combination was found that enabled subsequent penetrations to about 100 ft bgs, water sample collection, rod retraction, and hole closure to be achieved at a rate of about one hole per day. In the remainder of the demonstration, the only equipment difficulty encountered was a jammed, broken drill bit that resulted in about 6 hours of lost production.

A map of the UMCD Explosives Washout Lagoons and model of the contamination plume is presented in Figure 14. A total of six penetrations to groundwater were completed during the demonstration – the locations of those penetrations are indicated by green numbers on the map.

3.5.2 Residuals Handling

At both ALAAP and UMCD, disposal of the small quantities of water sample collected and analysis waste generated was handled by the on-site contractors. The major source of residuals in the demonstrations was soil cuttings generated during enhanced CPT air drilling operations. Although the enhanced CPT system can be configured to collect cuttings, we were permitted to discharge the directly to the ground surface until groundwater was reached. At the first sign of wet discharge we discontinued the air purge so that material was not forced to the surface. Therefore, the only waste generated by CPT operations was water used for rod decontamination which amounted to less than 50 gallons for the duration of the demonstration and was permitted to be disposed on the ground.

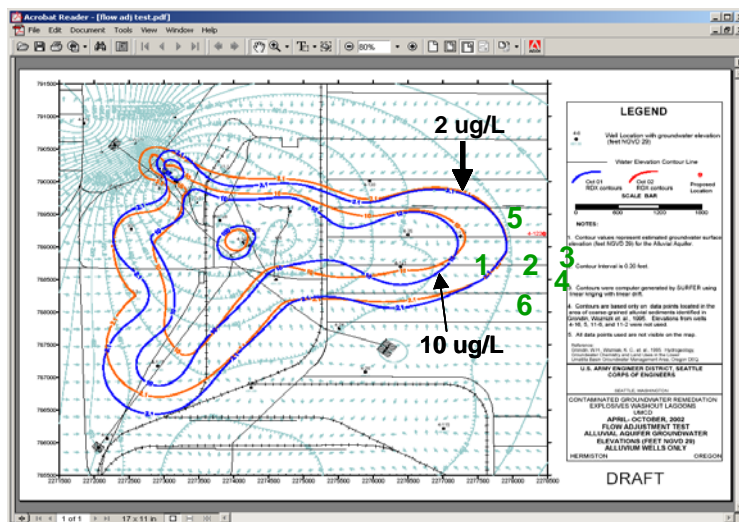


Figure 14. The Explosives Washout Lagoons at UMCD. CPT sampling locations are numbered 1-6 in green on the map.

3.5.3 Operating Parameters for the Technology

The SERS technology demonstrated in this project required a single operator. However, an unattended analyzer can be developed for remote process monitoring or other applications. As with most analytical techniques, such as HPLC and colorimetry, SERS measurements are performed sequentially. Batch mode processing of multiple samples is feasible with SERS, but offers only a modest cost and time advantage because sample collection and detector integration times are considerably longer than sample preparation time. Like for colorimetry, batch mode sample preparation (i.e., using a vacuum manifold) is an advantage when sample preconcentration by solid phase extraction is employed – passing large volumes of water through the filters and preconcentration cartridges can be time consuming, especially for turbid samples which quickly plug the 0.45 μm filters.

3.5.4 Experimental Design

In this demonstration our data quality objective was to validate the performance of the SERS method for quantifying explosives in groundwater samples. To achieve this goal, our experimental design called for split samples of groundwater to be analyzed by SERS, colorimetry (using the procedures in references 2 and 20), and EPA SW-846 Method 8330. Method 8330 is included as Appendix A to this report. The split-sample approach is commonly used to validate on-site methods and avoids the considerable uncertainties of sample-to-sample variation.³ Because water samples are inherently homogenized, subsampling errors common in soil analysis are also avoided.

Figure 15 is a flow chart summarizing the experiments performed on each water sample in the ALAAP demonstration. The samples were split and analyzed in the field using SERS and colorimetric procedures. Each procedure was performed using duplicate aliquots carried through the entire procedure, and triplicate when explosives were detected. SAIC also sent splits of the samples to an independent certified contract laboratory (IT Corp.) for Method 8330 analysis as prescribed in the site remedial investigation plan. The remainder of each sample was sent to ARA/CRREL for further split analysis by SERS, colorimetry, and HPLC.

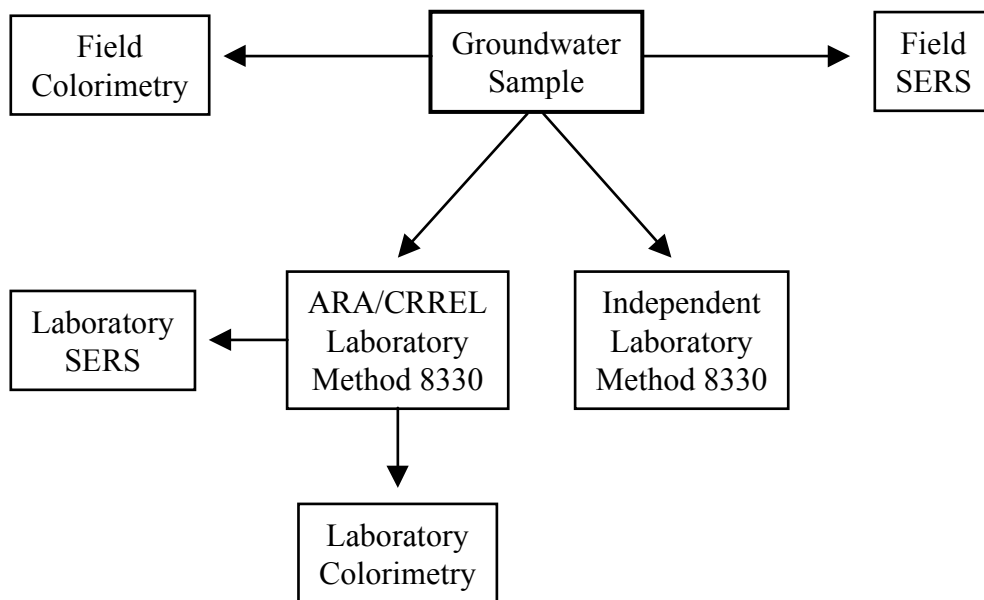


Figure 15. Experimental flow chart for analysis of split water samples.

A flow chart similar to Figure 15 applied to the GAC Plant process water samples except that the independent laboratory method specified by the site process monitoring plan was colorimetry for all three sampling events except the last, when the independent laboratory also performed Method 8330 analysis. For the CPT demonstration, the independent laboratory performed colorimetry and there was an additional analysis (*in situ* SERS) performed on each of the six groundwater samples.

The split sample approach described above minimized the effects of sampling and potential holding time variances, allowing actual *method* performances to be more reliably compared as described in the next section.

3.5.5 Sampling Plan

Collection of groundwater from wells at ALAAP and process water samples at the UMCD GAC Plant was coordinated with ongoing remedial investigation activities at the sites. Sampling was conducted by on-site contractors (SAIC and SCS Engineers) who are experts in this area and followed approved procedures for the sites delineated in their remedial investigation plans. CPT

water samples were collected by trained ARA personnel using a commercial pneumatic bladder pump (Durham Geo) lowered through the rod string. The pump and tubing were cleaned with DI water between samples. Receiving vessels were amber glass bottles with Teflon lined caps. However, as discussed in Section 3.5.3, this study was designed to avoid the high variability (uncertainty) of sampling by using field split samples for the comparison of methods. Thus, even if unrepresentative (inaccurate) samples were collected from the wells or process stream there was no impact on the validity of the results, since they did not rely on the absolute measured result for each sample.

Section 3.5.3, including Figure 15, describes the samples collected and split for analysis in each demonstration. Field and laboratory colorimetry followed standard procedures for the analysis of nitroaromatics and nitramines in water samples.^{2,20} SERS and Method 8330 analyses focused on the five primary analytes found at the sites – TNT, 2,4-DNT, TNB, RDX, and HMX. Other explosives were not detected.

The aforementioned on-site contractor sampling experts also handled the labeling, chain of custody, preservation, packing and shipping of samples to ARA/CRREL and the independent reference laboratories in accordance with procedures prescribed under Method 8330. All analyses were performed within 48 hrs of sample shipment from ALAAP, resulting in a maximum holding time of five days, which was within the seven days allowed under Method 8330. A trip DI blank was included with each shipment. The contractors did well to avoid sample damage or contamination – not one sample bottle leaked or broke in transit to Vermont and every trip blank tested clean. Additionally, reagent blanks were run at the beginning of each day for each field or laboratory method and also tested clean in all cases.

Quality assurance and control were consistent with the procedures outlined in Method 8330 for laboratory HPLC analysis and recommended for colorimetric field screening.^{3,20} In both the field and laboratory, the SERS response calibration was checked thrice daily with an intermediate concentration standard (150 µg/L prepared daily from a refrigerated stock solution) containing all relevant analytes. In all cases, the responses were within +/- 15% of the expected value obviating the need for re-calibration of the SERS instrument. Wavelength calibration of the SERS instrument was performed with naphthalene upon arrival at each demonstration and thereafter checked at the beginning and midpoint of each day – there was no change in calibration (within one detector pixel, or 1.8 cm⁻¹) observed throughout the project. Indeed, the initial calibration at each site was not required as there was no change from the laboratory calibration. Following recommended procedures,²⁰ colorimetry response calibration was checked at the beginning of each day with TNT and/or RDX control standards (2 mg/L). Again, all responses were within +/- 15% of expected values and therefore did not require a change in calibration factor. HPLC quality followed Method 8330. More specifically, calibration verifications were performed at the beginning of each day with an intermediate concentration standard (150 µg/L) mixture of all analytes. In the course of this project, all HPLC calibrations verified to within +/- 15% of the expected values. Retention time windows for each analyte were established at the beginning of each day and every 10 samples thereafter.

3.5.6 Statistical Methods and Hypothesis Testing

There are a number of statistical procedures that can be used to compare the analytical performance of the SERS method against the colorimetric and reference HPLC methods. As pointed out by Crockett et al,³ in most cases measures of precision and bias are determined. Precision refers to the agreement among a set of replicate measurements and is commonly reported as relative percent difference, relative standard deviation, or the coefficient of variation. Bias relates to accuracy and refers to systematic deviation from the "true" value. A detailed discussion of precision and accuracy (bias), including calculations relevant to this project, can be found in Section B.3 in the QAPP (Appendix B).

This project generated data pairs for each sample (e.g., SERS and Method 8330). As such, this allowed paired statistical tests such as t-tests to be performed to compare methods. A useful framework for using paired tests to compare analytical methods is null hypothesis testing, whereby the claim that there is no statistically significant difference between the new (e.g., SERS) methodology and the reference (e.g., Method 8330) methodology is tested. More specifically, the null hypothesis (H_0) can be stated as "the mean of the population of differences between the two analytical methods is zero ($\mu=0$).". The null hypothesis can be evaluated against the alternative hypothesis ($H_0:\mu\neq0$) that the mean difference is greater than or less than zero. On the basis of the random sample from the population, one decides whether to accept or reject the null hypothesis.

Both parametric and non-parametric tests exist for the purpose of hypothesis testing, and the applicability of each type depends on the distribution of the population, as inferred from the distribution of the random sample obtained. The Student test is a parametric test of paired data used to test hypotheses about the mean of a population. The Student t test is only applicable to a population that is near normal or can be transformed to a normal distribution. In cases where the population of differences is not normally distributed, and the differences of log concentrations are also not normally distributed, a non-parametric test should be performed. The Wilcoxon Signed Rank test, also known as the Wilcoxon Matched Pairs test, is the most powerful non-parametric test and applies if the population is symmetric. If the population is asymmetric, a Sign test can be performed.

The process of hypothesis testing begins with calculating paired differences by subtracting the reference method result from the new method (e.g., SERS) result. Next, assumptions about the normality of the distributions of paired differences are tested by application of the Shapiro-Wilk W test or comparable test (e.g., Ryan-Joiner test). For example, at a 90% confidence, the two-tailed Shapiro-Wilk W test will reject the assumption that the data are normally distributed when the p-value associated with the W is less than 0.05. Normality testing was performed on the paired difference data set for each analyte of interest. In cases where the assumption of normality held (i.e., $p > 0.05$), a one-sample t test was then be applied to test the null hypothesis that the mean of the differences was equal to zero ($H_0:\mu=0$) against the alternative hypothesis that the mean was not equal to zero ($H_0:\mu\neq0$).

In cases where the assumption of normality did not hold (i.e., $p < 0.05$), then the differences of the logarithms of the results was calculated, and the test of normality applied to these data as described above. If the differences of the logarithms was found to be normally distributed ($p > 0.05$), then the one-sample t test was applied to test the null hypothesis that the mean of the differences of the logarithms was equal to zero. In this case, since the null hypothesis is tested using the difference of logarithms, it is equivalent to testing that the ratio of the two analytical results is equal to one.

If neither the differences nor the differences of the logarithms of the analytical results were found to be normally distributed for any analyte, then the non-parametric Wilcoxon Signed Rank test was applied to the differences, testing the null hypothesis that the median of the differences is equal to zero against the alternative hypothesis that the median is not equal to zero.

The analytical results included non-detects. When using the parametric t test, pairs of two non-detects were dropped from the sample, reducing N accordingly. For paired results containing one non-detect, it was replaced by half the analytical detection limit. For the non-parametric Wilcoxon Signed Rank test, pairs of two non-detects were dropped from the sample, again reducing N accordingly. Pairs containing one non-detect were ranked according to the difference between the quantified result and half the detection limit for the Wilcoxon Signed Rank test.

Crockett et al³ assert that when the concentrations of explosives cover a large range of values, regression methods for assessing precision and accuracy become appropriate. This is because as the variability in the sample concentration increases, the capability for the paired tests described above to detect differences in precision or bias decreases. Regression analysis is useful because it allows characterization of non-constant precision and bias effects and is normally performed with the reference analytical method (e.g., Method 8330) result as the independent variable. The concentrations measured at ALAAP and UMCD covered a wide range of values (low $\mu\text{g/L}$ to mg/L); therefore we applied regression analysis to the data as discussed in Section B.3 in the QAPP (Appendix B).

3.5.7 Demobilization

Packing up and demobilizing from the demonstration sites required approximately the same amount of time as mobilizing and setting up at the sites. The SERS and colorimetry field analysis equipment was packed up in about an hour. After all of the CPT holes were abandoned in accordance with OAR 690-220 regulations at UMCD, the CPT truck and equipment were packed and returned to Richland, WA in about 3 hrs.

3.6 Selection of Analytical/Testing Methods

As discussed previously, a major objective of this project was to compare the analytical performance of the in-house SERS technology against EPA SW-846 Method 8330, "Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC)" and the colorimetric procedures developed by Jenkins and co-workers² and commercialized by Strategic

Diagnostics²⁰ for explosives in groundwater. Method 8330, included as Appendix A to this report, is the most widely used laboratory method for analyzing explosives and colorimetry is the predominant field screening method for explosives.

4. Performance Assessment

4.1 Performance Criteria

Table 3. Performance Criteria

Performance Criteria	Description	Primary or Secondary
Analytical Performance	1. SERS MDL in low µg/L range 2. Resolution of at least 3 analytes.	Primary
Process Waste	Less than 5 mL of waste generated per sample. The waste is no more hazardous than the groundwater sample itself, which poses no explosive or acute toxicity hazard.	Primary
Factors Affecting Technology Performance	1. Groundwater pH. Sample pH outside the range 3-12 can affect analytical performance, requiring that pH adjustment be performed prior to analysis. 2. Sample turbidity. As with all optical based techniques, turbid samples must be filtered to avoid attenuation of the response.	Secondary
Fieldability and Ease of Use	SERS requires a single person to perform the test. Our performance objective was for the operator to prepare and analyze a sample in 15 min or less. The level of expertise needed to perform the analysis is comparable to that required for the colorimetric field methods. This level of expertise is often understated; in reality, a technician with chemical handling skills is required to perform the method accurately, reproducibly, and safely. Special OSHA training is not required, but is recommended for non-chemists and may be required at many field sites. For <i>in situ</i> SERS, our goal was to demonstrate a minimum of operator involvement – i.e., to initiate the analysis and interpret results only.	Primary
Versatility	The SERS method can be used at virtually any site contaminated with explosives. The groundwater method can be adapted for soil analysis at sites where contaminants have not yet reached the water table.	Secondary
Maintenance	There is no routine maintenance required for the SERS instrument which should provide years of service. Raman instrumentation is best returned to the factory for repair. If the end of the fiber optic probe becomes dirty, as indicated by a loss of optical power (weak response), it can be simply cleaned with alcohol on lens tissue. Sampling equipment requires periodic cleaning.	Secondary
Scale-Up Constraints	None.	Secondary

4.2 Performance Confirmation Methods

Adherence to the Experimental Design and Sampling Plans described in Sections 3.5.3 and 3.5.4 and the QAPP (Appendix B) ensured that reliable data was collected and a valid comparison of methods could be performed. The data quality parameters comparability and representativeness were ensured by the use of split samples throughout the project. Sufficient data was collected to evaluate the SERS performance criteria listed in Table 4. Our data completeness goal for the project was 90% with a minimum of 30 total valid samples. This goal was met with over 50 samples collected, all of which were valid.

Table 4. Expected Performance and Performance Confirmation Methods

Performance Criteria	Expected Performance Metric	Performance Confirmation Method	Actual
Primary Criteria – Qualitative			
Fieldability	Instrument can be used in the field	Experience from demo operations	Five fieldings without problems
Ease of use	Comparable to colorimetry	Experience from demo operations	Sample preparation simpler than colorimetry; instrumentation slightly more complex
Primary Criteria – Quantitative			
Analytical performance (MDL, range)	Low µg/L to >100 µg/L	EPA Method 8330 (see Appendix A)	MDL 2.6 to 5.1 µg/L; linear range to 500 µg/L
Spectral resolution	3 analytes or more	EPA Method 8330	Resolved 4 analytes in real-world samples
Speed	<15 min/sample	Observation/timing	7-12 min/sample
Process waste	<5 mL/sample	Observation/volumetric measurement	1-3 mL/sample
Secondary Criteria			
Factors affecting performance	pH no effect outside the range pH 3-12; turbidity no effect with filtering	Measure pH and filter samples; EPA Method 8330	All samples within 0.5 pH units of neutral (pH 7)
Safety	Gloves and eye protection	Experience from demo operations	No safety issues, gloves and eye protection used when handling samples or reagents
Versatility	Useful at other sites	Experience from demo operations	General utility for groundwater and process water monitoring demonstrated
Maintenance	None	Experience from demo operations	No maintenance required
Scalability constraints	None	Experience from demo operations	No interferences or matrix effects observed

4.3 Data Analysis, Interpretation and Evaluation

Data analysis, interpretation, and evaluation followed the procedures set forth in Sections 3.5.3-3.5.5 and the QAPP (Appendix B). The remainder of this section reports the results of our data analysis, interpretation and evaluation relating to the performance of the SERS method and its comparison with Method 8330 and colorimetry. Analytical results for the water samples included in this study are compiled in Appendix C.

4.3.1 Basic Analytical Performance

Fundamental performance parameters established for the SERS method were the MDL, linear dynamic range, accuracy (measured as percent recovery of spiked samples), and precision (measured as percent relative standard deviation for replicate measurements). The linear dynamic range for SERS analysis of explosives is from the lower detection limit (ca 5 $\mu\text{g/L}$) to approximately 500 $\mu\text{g/L}$ as shown in the calibration curve for TNT presented in Figure 16. The upper limit of the linear range exceeded our performance goal of 100 $\mu\text{g/L}$.

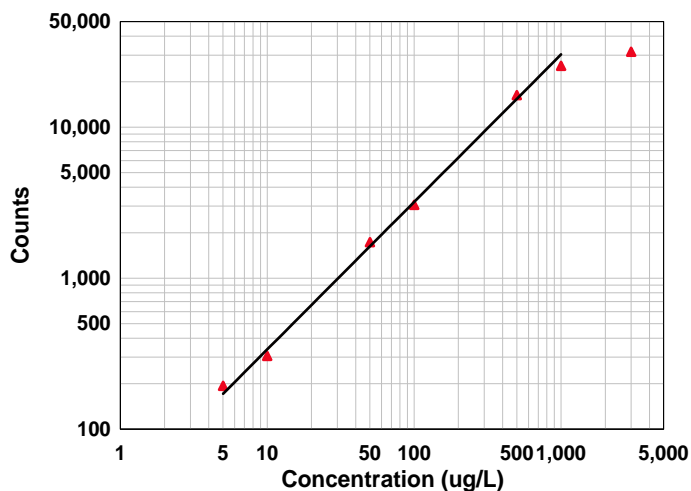


Figure 16. SERS linear calibration curve for TNT in water.

MDLs were determined with a set of eight replicate samples for the five explosives detected in the demonstrations at ALAAP and UMCD. Samples spiked at 15 $\mu\text{g/L}$ in clean, filtered ALAAP groundwater matrix were used for the MDL test – 15 $\mu\text{g/L}$ was three times the estimated MDL of 5 $\mu\text{g/L}$. The SERS MDLs are presented in Table 5 along with MDLs for the HPLC and colorimetric methods. Our SERS performance goal of low $\mu\text{g/L}$ MDLs was met; the MDLs are low enough to screen at all drinking water health advisory levels except Lifetime levels of 2 $\mu\text{g/L}$ for RDX and TNT.¹¹ In the case of the nitramines, a 10-fold sample preconcentration using established solid phase extraction procedures^{2,8} was required to achieve the desired sensitivity. However, for colorimetry to reach a comparable MDL, a nearly 300-fold preconcentration (2 L water sample) was required. Without preconcentration, HPLC Method 8330 was consistently most sensitive. Because capability for preconcentration was not part of our CPT SERS probe,

the MDL for RDX was well above groundwater concentrations, thereby obviating the effectiveness of *in situ* SERS at the site.

Table 5. Method Detection Limits (µg/L)

Analyte	SERS	HPLC	Colorimetry
2,4-DNT	3.8	2.0	0.7*
TNT	4.3	1.0	
TNB	5.7	0.5	
RDX	2.6*	1.0	2.8*
HMX	5.1*	1.0	

* With sample preconcentration

Table 6 summarizes the accuracy and precision of SERS vs. HPLC and colorimetry. For nitroaromatics, SERS and HPLC had comparable accuracies. The accuracy of HPLC was superior for nitramines for which the SERS method is not as sensitive. The accuracies of the two colorimetric methods were lower than SERS and HPLC, especially for TNT which was reported to exhibit a low recovery of 79%.² HPLC Method 8330 precision was consistently better than SERS and colorimetry. However, the single-laboratory precision of SERS was better than multilaboratory HPLC precision reported in Method 8330. SERS precision was better for nitroaromatics than nitramines. This is not surprising considering the additional error introduced by the preconcentration step used with the nitramines. Further optimization of the preconcentration step could potentially improve the precision for nitramines by 1-2% RSD. The preconcentration step may also account for the lower SERS recovery of HMX. Once again, the reported precision for colorimetry was not as favorable as the other methods, especially for nitramines (RDX, 26.8% RSD).

Table 6. Accuracy and Precision of Analytical Methods

Analyte	Accuracy			Precision			
	SERS % Recovery	HPLC* % Recovery	Color** % Recovery	SERS %RSD	HPLC*** %RSD	Color** %RSD	HPLC Multilab* %RSD
2,4-DNT	96.7	98.6	---	3.4	0.6	---	7.2
TNT	94.7	94.4	79.4	3.8	0.4	7.3	10.4
TNB	91.9	---	---	4.7	1.6	---	---
RDX	93.3	99.6	91.2	5.0	0.9	26.8	7.6
HMX	89.3	95.5	---	5.8	2.1	---	7.3

* From SW-846 Method 8330 (Appendix A)

** From reference 2

*** From reference 21

Overall, basic analytical SERS performance met the objectives of this project and was not far from the performance of the reference laboratory method. In general, SERS performance exceeded the colorimetric methods for nitramine and nitroaromatic explosives.

4.3.2 Statistical Analysis

Linear regression analysis and hypothesis testing using paired statistics formed the basis for much of our statistical comparison of SERS analytical performance vs. the other two methods. The goals of this effort were to answer the following questions:

- (2) Is field SERS an analytically acceptable alternative to HPLC or colorimetry for the analysis of explosives in water?
- (3) Is the performance of SERS in the field comparable to laboratory SERS?
- (4) Are there any significant performance differences for different explosive analytes?

Results from our paired statistical tests of the null hypothesis performed at the 90% confidence level are summarized in Table 7. The key metric in the table is the P value, which relates to the probability of being wrong if the null hypothesis is rejected. At P values above 0.1 the null hypothesis is accepted.

The first four lines in Table 7 resulted in the acceptance of the null hypothesis, answering the second question above in the affirmative (the performance of field SERS is comparable to laboratory SERS). The null hypothesis was accepted for individual and combined analytes. This result was expected because there were no procedural differences between the field and laboratory methods and the Raman instrument performance was stable in the field. In the cases of the combined and TNT results, the non-parametric Wilcoxon test was narrowly passed. Statistical power testing at the P-value of the test (see discussion of power testing below) gave powers of 0.615 and 0.531 for combined and TNT results, respectively. These values are sufficiently large to give us confidence in accepting the null hypothesis (i.e., that we would correctly reject a false null hypothesis). The second group of data (lines 4-7) similarly results in acceptance of the null hypothesis in all cases, indicating that the performance of field colorimetry was comparable to laboratory colorimetry. Again, these results were expected for the same reasons as for SERS.

The third group of data, comparing field and laboratory SERS with Method 8330, also resulted in the acceptance of the null hypothesis in all cases for both individual and pooled analytes. These results were significant in that they answer the first question above in the affirmative (i.e., SERS is an acceptable alternative to Method 8330 for the analysis of explosives in water samples, especially the most prevalent explosive contaminants RDX, TNT, and 2,4-DNT). However, regulatory acceptance of the SERS method is not concerned with minimizing the probability of a Type I error (e.g., incorrectly rejecting a correct null hypothesis). Therefore, one can maximize the *power* of a test of hypothesis (e.g., the probability of correctly rejecting a false null hypothesis) by accepting a significance level, α , equal to the P-value of the test for the given random sample. Table 8 presents power values for SERS data against Method 8330 data resulting from this type of analysis. These power values indicate the probability with which we would correctly reject a false null hypothesis, based on the random sample of data obtained. The power values for combined analytes for both field and laboratory SERS are large, lending further validity to our acceptance of the null hypothesis and indicating appropriateness of the SERS

method for regulatory approval. The power values show no significant difference between individual analytes when average field and laboratory values are compared.

Table 7. Results of Paired Statistical Testing of Analytical Methods

Difference Compared*	Analyte	N	Mean (ug/L)	Std. Dev. Mean (ug/L)	Median (ug/L)	Std. Dev. Median (ug/L)	K-S Normal Test P	Paired t Test P	K-S Normal Test on Logs P	Paired t Test on Logs P	Wilcoxon Matched Pairs Test P
LSER-FSER	Combined	51	22.3	83.8	1.0	29.2	<0.01		<0.01		0.111
LSER-FSER	RDX	12	0.4	3.2	0.0	3.9	>0.15	0.661			
LSER-FSER	2,4-DNT	19	47.3	126.8	0.0	132.1	<0.01		>0.15	0.524	
LSER-FSER	TNT	14	16.9	56.1	2.0	56.1	<0.01		0.025		0.140
LColor-FColor	Combined	32	-23.9	87.2	-1.5	88.7	<0.01		>0.15	0.755	
LColor-FColor	Nitramines	9	-0.2	5.1	-1.0	4.9	>0.15	0.900			
LColor-FColor	Nitroarom.	23	-33.2	101.9	0.0	105.1	<0.01		>0.15	0.776	
LC-FSER	Combined	57	33.2	122.2	0.0	125.4	<0.01		<0.01		0.199
LC-FSER	RDX	13	0.2	5.1	0.0	4.9	>0.15	0.916			
LC-FSER	2,4-DNT	20	69.4	171.6	1.5	180.5	<0.01		>0.15	0.792	
LC-FSER	TNT	15	33.6	127.2	1.0	127.1	<0.01		<0.01		0.589
LC-LSER	Combined	57	13.2	53.8	0.0	53.9	<0.01		<0.01		0.546
LC-LSER	RDX	13	-0.8	4.5	-1.0	4.3	>0.15	0.510			
LC-LSER	2,4-DNT	20	24.5	63.4	-1.0	66.8	<0.01		>0.15	0.480	
LC-LSER	TNT	15	17.9	74.9	-2.0	75.0	<0.01		<0.01		0.875
LC-LColor	Combined	32	55.4	191.2	0.5	196.0	<0.01		>0.15	0.978	
LC-LColor	Nitramines	9	-1.2	5.6	-2.0	5.3	0.075	0.528			
LC-LColor	Nitroarom.	23	77.6	222.9	4.0	230.0	<0.01		>0.15	0.213	
FColor-FSER	Combined	32	28.5	85.3	2.0	88.0	<0.01		>0.15	0.325	
FColor-FSER	Nitramines	9	3.6	5.4	3.0	5.1	>0.15	0.082			
FColor-FSER	Nitroarom.	23	38.2	99.4	0.0	104.5	<0.01		>0.15	0.785	
LColor-LSER	Combined	32	-31.0	125.0	-0.5	126.7	<0.01		>0.15	0.888	
LColor-LSER	Nitramines	9	2.6	3.0	2.0	2.8	>0.15	0.032			
LColor-LSER	Nitroarom.	23	-44.1	146.2	-1.0	149.3	<0.01		>0.15	0.087	
RLC-FSER	Combined	23	106.0	356.1	-3.0	365.0	<0.01		>0.15	0.029	
RLC-LSER	Combined	23	60.6	307.7	-4.0	307.8	<0.01		>0.15	0.046	
RLC-LC	Combined	23	27.8	246.8	-2.0	243.2	<0.01		0.046		0.132
RLC-FColor	Combined	13	127.2	586.5	-8.0	579.5	<0.01		>0.15	0.103	
RLC-LColor	Combined	13	182.2	621.3	-2.0	624.7	<0.01		>0.15	0.084	
RColor-FSER	Combined	13	4.7	8.1	3.0	8.0	>0.15	0.059			
RColor-LSER	Combined	13	3.9	7.3	2.0	7.2	0.068	0.075			
LC-RColor	Combined	13	-3.0	9.3	-2.0	9.0	>0.15	0.269			
RColor-FColor	Combined	13	2.0	7.4	3.0	7.2	<0.01	0.351			
RColor-LColor	Combined	13	2.3	6.3	2.0	6.0	>0.15	0.209			

* L=Laboratory; F=Field; SER=SERS; LC=HPLC; R=Reference Laboratory; Yellow highlighted values reject the null hypothesis

Table 8. Statistical Power Values for SERS vs. Method 8330

Difference Compared	Analyte	N	Power
LC-FSER	Combined	57	0.774
LC-FSER	RDX	13	0.917
LC-FSER	2,4-DNT	20	0.799
LC-FSER	TNT	15	0.743
LC-LSER	Combined	57	0.901
LC-LSER	RDX	13	0.588
LC-LSER	2,4-DNT	20	0.582
LC-LSER	TNT	15	0.918

The fourth set of data in Table 7 also leads to acceptance of the null hypothesis in all cases, indicating that, like SERS, the color methods are also suitable (approvable) for the analysis of explosives in water samples. These results confirm those of previous studies.^{2,8} Field color results were similar to the laboratory color results reported in Table 7.

SERS and colorimetry are compared in the fifth data set. The null hypothesis is accepted for the pooled data, but is rejected for three of the four remaining comparisons of explosive classes. The data indicate poorer agreement between the two less precise field methods than either field method compared against the more precise Method 8330. Nitramines demonstrated the poorest agreement and were biased higher in the color method.

The sixth and seventh data sets compare reference laboratory results against the ARA/CRREL field and laboratory results by all three methods. For the reference laboratory performing Method 8330 (nearly all ALAAP sample data), the null hypothesis was rejected in three out of five cases. In the other two cases (comparison against field colorimetry and our Method 8330) the null hypothesis was narrowly accepted ($P=0.103$ and 0.132 , respectively). As is documented in Method 8330 and is generally well-known, multi-laboratory precision is poorer than for a single laboratory (see Table 6). However, the relatively poor agreement with three methods (one of which was also Method 8330) that agreed far better with each other (see previous discussion of Table 7) suggests error in the reference laboratory results. This is further supported by the fact that the reference laboratory results were consistently biased high relative to the other methods. The reference laboratory colorimetry results (from UMCD) compared better, leading to acceptance of the null hypothesis except when compared against SERS. This would not be surprising, given the generally lower agreement between the two field methods (see discussion for the fifth data set above). Again, consistent with the rest of the data, there was no significant difference in comparative performance for the individual analytes.

The wide range of concentrations encountered in this study support the use of linear regression analysis for the comparison of methods. Table 9 is a summary of linear regression results, with the method comparisons grouped as in Table 7. Overall, the linear regression statistics support the conclusions reached in the paired statistical analysis. The first two data sets indicate that the field and laboratory results for both SERS and colorimetry were comparable. Correlation

coefficients and slopes were near 1.0 (although the differences from 1.0 were statistically significant at the 95% confidence level – the lower and upper 95% values did not bracket 1.0 in six out of eight cases) and intercepts were near zero (in all cases the upper and lower 95% confidence values bracketed a zero intercept). Performance for nitramines was less ideal than for the nitroaromatics, but this was likely due to the high percentage of low concentration (near detection limits) nitramine samples. The greater scatter in the data at low concentrations is evident in Figure 17, which is a plot of colorimetry and SERS field vs. laboratory results. The graph also shows the nearly equal distribution of results above and below the ideal line, which further indicates little or no bias in the data.

SERS data compared favorably with the laboratory Method 8330 results in the third data set. Again, the greatest scatter in the data was for the nitramine RDX in both the field and the laboratory although overall intercepts were near zero and slopes were near, but slightly less than 1.0. This small, but statistically significant, low bias in the slope is expected when there is error in the reference method (i.e., the “true” value).²² Figure 18 is a graph of the pooled SERS (i.e., combined field and laboratory) vs. HPLC results. As with the previous graph, there is increased scatter at low concentrations, where most of the RDX data points reside. A slope slightly less than 1.0 is also evident. The graph of pooled colorimetry results vs. HPLC results (Figure 19) is very similar to Figure 18, except the scatter is greater (especially for nitramines) and the slope is even lower, with an intercept deviating on the positive side of zero. These observations are confirmed quantitatively in the fourth data set of Table 9 and suggest some positive bias in the colorimetric method, perhaps due to positive sample matrix interferences. The fifth data set and Figure 20 reveal high correlations between pooled SERS and colorimetry methods - again with more scatter for the nitramines.

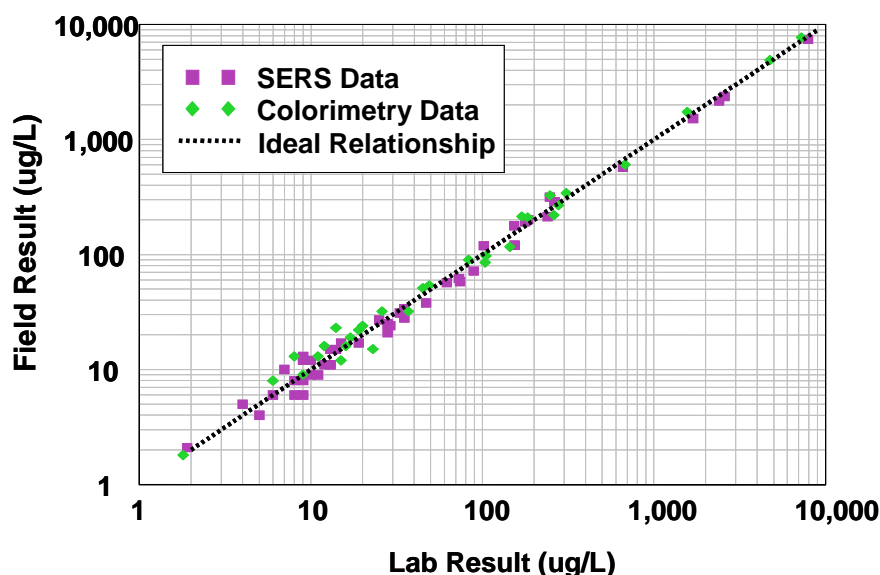


Figure 17. Comparison of SERS and colorimetry field and laboratory results.

Table 9. Linear Regression Analysis of Analytical Methods

Y	X	Analyte(s)	N	R-sqrd	Std Err	Res. Var.	Intcpt	Lower 95%	Upper 95%	{ 0 }	Slope	Lower 95%	Upper 95%	{ 1 }
FSER	LSER	Combined	51	0.9996	23.8	4.88	0.46	-6.50	7.42	Yes	0.933	0.928	0.939	No
FSER	LSER	RDX	12	0.9342	2.9	1.72	2.15	-0.97	5.28	Yes	0.793	0.644	0.941	No
FSER	LSER	2,4-DNT	18	0.9996	35.1	5.93	2.51	-16.16	21.18	Yes	0.935	0.926	0.945	No
FSER	LSER	TNT	13	0.9591	17.0	4.12	-0.22	-14.93	14.49	Yes	0.971	0.838	1.105	Yes
FColor	LColor	Combined	31	0.9994	38.4	6.20	-3.46	-18.43	11.50	Yes	1.053	1.043	1.062	No
FColor	LColor	Nitramines	8	0.8731	5.3	2.31	-3.17	-14.19	7.86	Yes	1.119	0.693	1.545	Yes
FColor	LColor	Nitroarom.	22	0.9994	46.1	6.79	-4.30	-26.59	18.00	Yes	1.053	1.041	1.065	No
FSER	LC	Combined	52	0.9978	51.9	7.20	-2.02	-16.97	12.93	Yes	0.907	0.895	0.919	No
FSER	LC	RDX	12	0.7896	5.3	2.30	1.46	-4.65	7.57	Yes	0.876	0.557	1.194	Yes
FSER	LC	2,4-DNT	18	0.9986	68.6	8.28	-5.03	-41.56	31.50	Yes	0.917	0.898	0.935	No
FSER	LC	TNT	13	0.9728	13.8	3.72	2.77	-8.96	14.50	Yes	0.949	0.844	1.055	Yes
LSER	LC	Combined	50	0.9987	43.9	6.63	-3.68	-16.62	9.26	Yes	0.971	0.961	0.982	No
LSER	LC	RDX	12	0.8908	4.6	2.15	-1.35	-6.71	4.02	Yes	1.134	0.854	1.414	Yes
LSER	LC	2,4-DNT	18	0.9993	53.1	7.29	-8.16	-36.45	20.12	Yes	0.980	0.966	0.994	No
LSER	LC	TNT	13	0.9900	8.4	2.91	3.98	-3.17	11.14	Yes	0.965	0.901	1.030	Yes
LColor	LC	Combined	31	0.9992	44.6	6.67	7.79	-9.55	25.13	Yes	0.890	0.880	0.899	No
LColor	LC	Nitramines	8	0.7978	6.2	2.49	2.67	-8.16	13.50	Yes	0.913	0.454	1.372	Yes
LColor	LC	Nitroarom.	22	0.9991	53.4	7.31	9.98	-15.79	35.74	Yes	0.889	0.877	0.901	No
FSER	FColor	Combined	31	0.9992	43.9	6.63	-3.32	-20.42	13.78	Yes	0.953	0.943	0.963	No
FSER	FColor	Nitramines	8	0.9203	3.0	1.72	3.93	-1.00	8.86	Yes	0.651	0.460	0.842	No
FSER	FColor	Nitroarom.	22	0.9992	52.8	7.26	-3.94	-29.43	21.54	Yes	0.953	0.941	0.966	No
LSER	LColor	Combined	31	0.9995	39.0	6.24	-9.97	-25.16	5.21	Yes	1.079	1.070	1.089	No
LSER	LColor	Nitramines	8	0.9587	2.8	1.68	-1.72	-6.73	3.29	Yes	0.987	0.782	1.191	Yes
LSER	LColor	Nitroarom.	22	0.9994	46.6	6.83	-13.03	-35.56	9.50	Yes	1.080	1.068	1.092	No
FSER	RLC	Combined	14	0.9529	498.9	22.34	34.51	-288.04	357.06	Yes	0.828	0.712	0.944	No
LSER	RLC	Combined	14	0.9590	500.2	22.37	30.11	-293.29	353.51	Yes	0.893	0.777	1.009	Yes
LLC	RLC	Combined	14	0.9742	412.7	20.32	32.49	-234.35	299.32	Yes	0.936	0.840	1.032	Yes
FColor	RLC	Combined	12	0.9598	518.2	22.76	45.52	-331.84	422.87	Yes	0.870	0.745	0.996	No
LColor	RLC	Combined	12	0.9652	457.8	21.40	45.13	-288.23	378.49	Yes	0.828	0.717	0.939	No
FSER	RColor	Combined	12	0.7208	6.4	2.54	4.42	-4.24	13.07	Yes	0.710	0.399	1.021	Yes
LSER	RColor	Combined	12	0.8275	4.3	2.06	6.24	0.52	11.95	No	0.639	0.433	0.844	No
LLC	RColor	Combined	12	0.5716	7.0	2.64	7.96	-0.61	16.53	Yes	0.520	0.203	0.837	No
FColor	RColor	Combined	12	0.7310	6.5	2.54	5.45	-2.48	13.38	Yes	0.687	0.393	0.980	No
LColor	RColor	Combined	12	0.8476	4.3	2.08	5.57	0.27	10.86	No	0.657	0.460	0.853	No

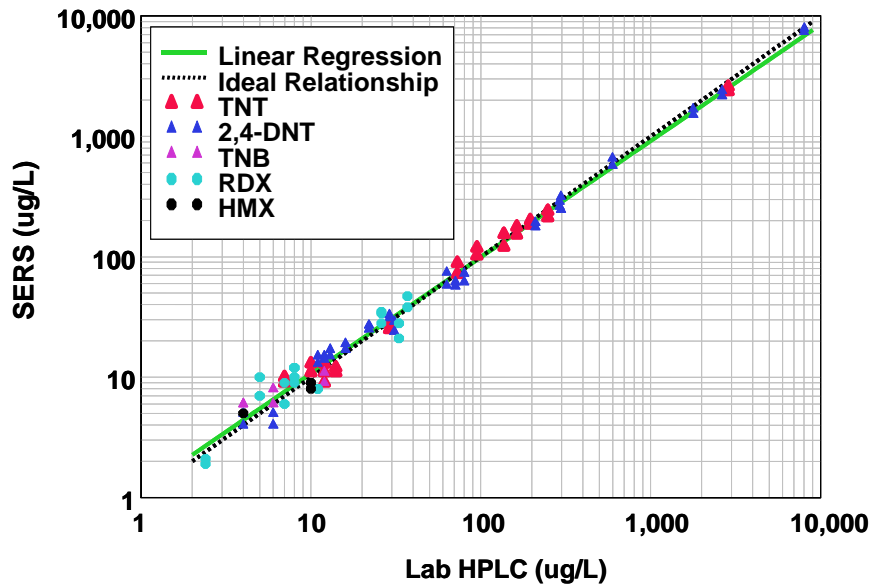


Figure 18. Comparison of SERS and Method 8330 results.

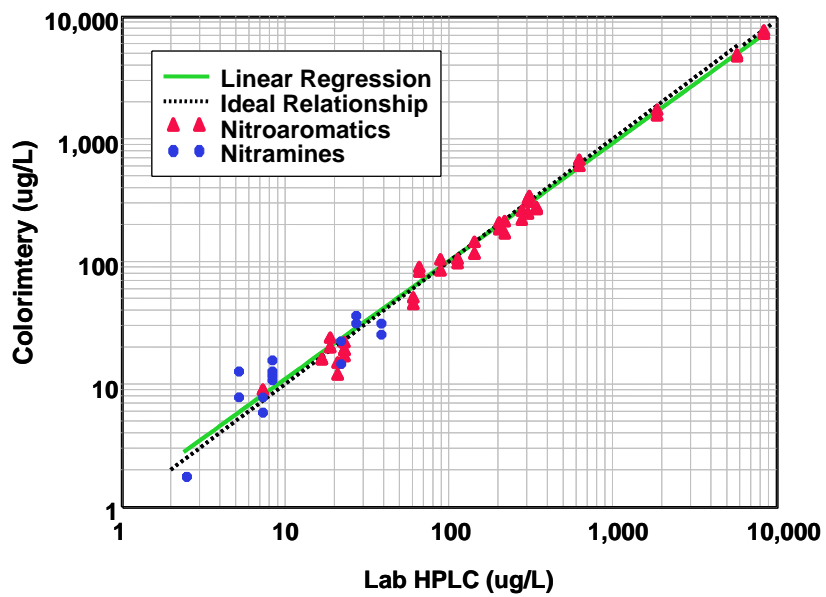


Figure 19. Comparison of colorimetry and Method 8330 HPLC results.

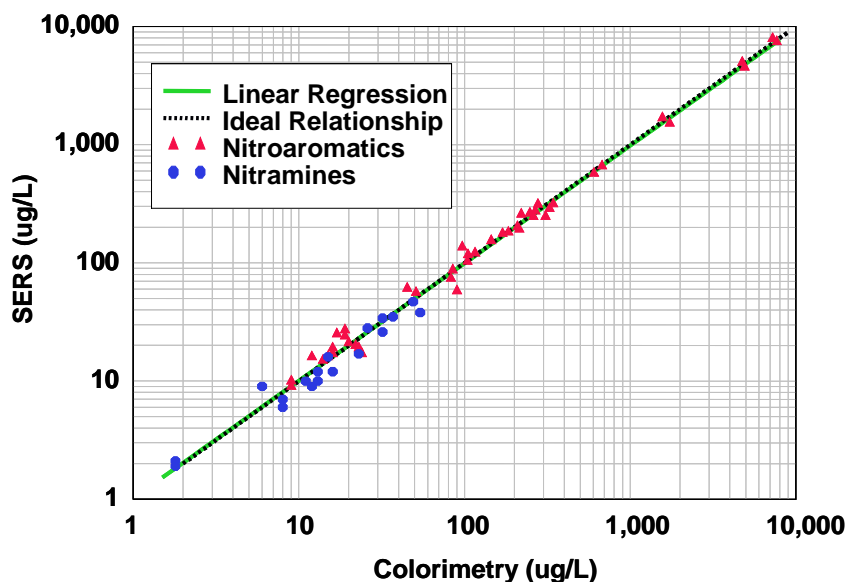


Figure 20. Comparison of SERS and colorimetry results.

As with the paired statistics, the poorest agreement between methods was observed when comparisons were made against the outside reference laboratory methods. Although data in the sixth set in Table 9 (comparison against reference laboratory HPLC) show reasonable correlation to the best fit line (correlation coefficients ca. 0.96), the slopes were consistently low with large positive intercepts. Figures 21-23 are graphs of laboratory HPLC, pooled SERS, and pooled colorimetry against the reference laboratory HPLC results for nitroaromatics. The similarity of the plots, showing significant positive bias for the linear regression vs. the ideal relationship in all cases, supports our paired statistics-based assertion that there was error in the reference laboratory results.

The seventh data set in Table 9 showed the weakest correlation to the best fit line (correlation coefficients of 0.8 and less) and low slopes (ca 0.6). Intercepts were consistently above, but not far from zero and in two cases did not bracket zero at 95% confidence. As shown in Figures 24-26 for reference laboratory colorimetry comparisons against pooled SERS, pooled colorimetry, and laboratory HPLC, the degree of scatter in the data is noticeably greater for nitramines than in previous plots (there is comparatively little data for nitroaromatics) that have markedly better regression statistics. The greater scatter is not surprising given the lower precision of the colorimetric methods^{2,8,22} being used as the reference methods and the lower precision and accuracy of inter-laboratory measurements. Lower precision in the reference method also results in lower regression slopes.²² Also of importance is the small range of concentrations range for UMCD samples, where the reference laboratory colorimetry was used – nearly two orders of magnitude smaller than at ALAAP. Indeed, most of the UMCD data was at low concentrations

where scatter is greater. The use of regression methods is most appropriate when analyte concentrations cover a large range of values.

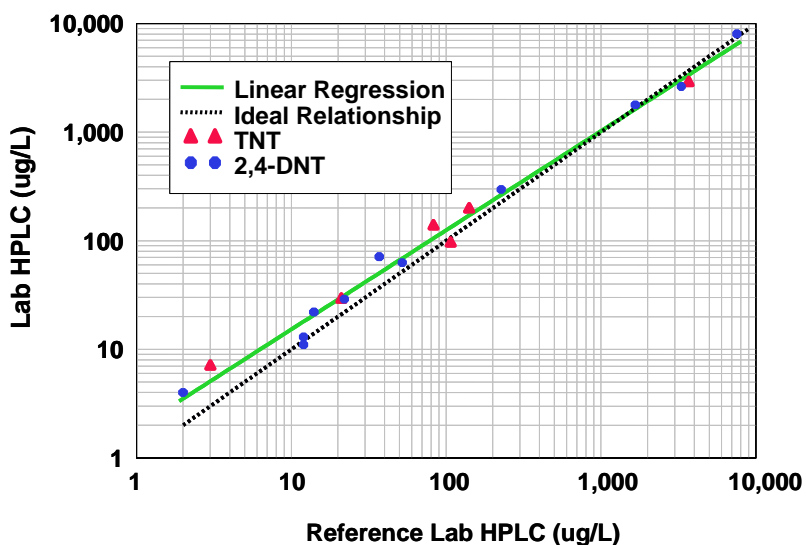


Figure 21. Comparison of laboratory HPLC and reference laboratory HPLC results.

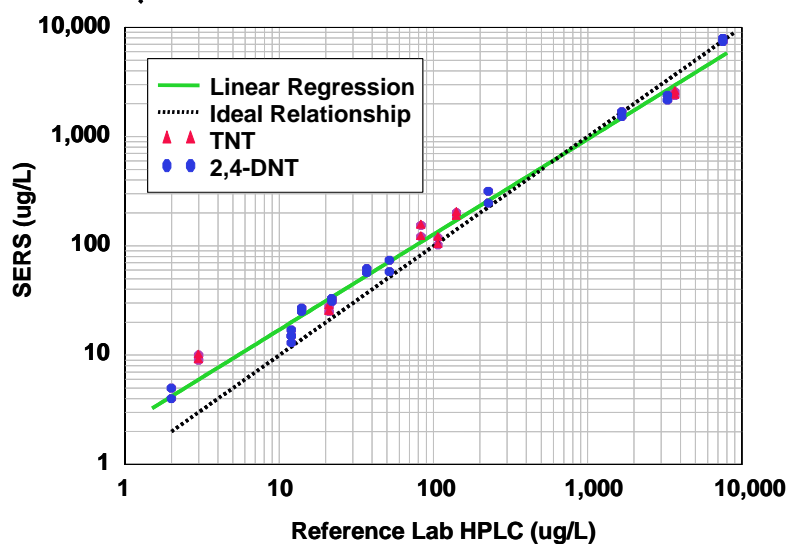


Figure 22. Comparison of SERS and reference laboratory HPLC results.

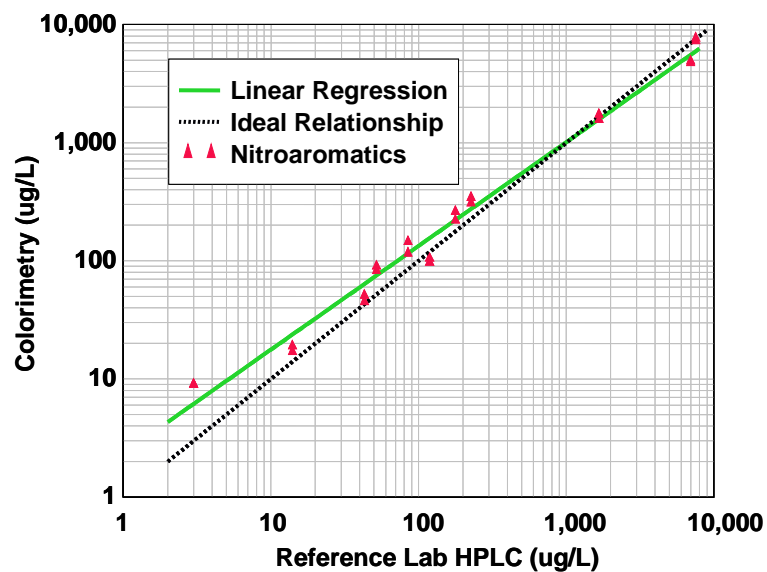


Figure 23. Comparison of colorimetry and reference laboratory HPLC results.

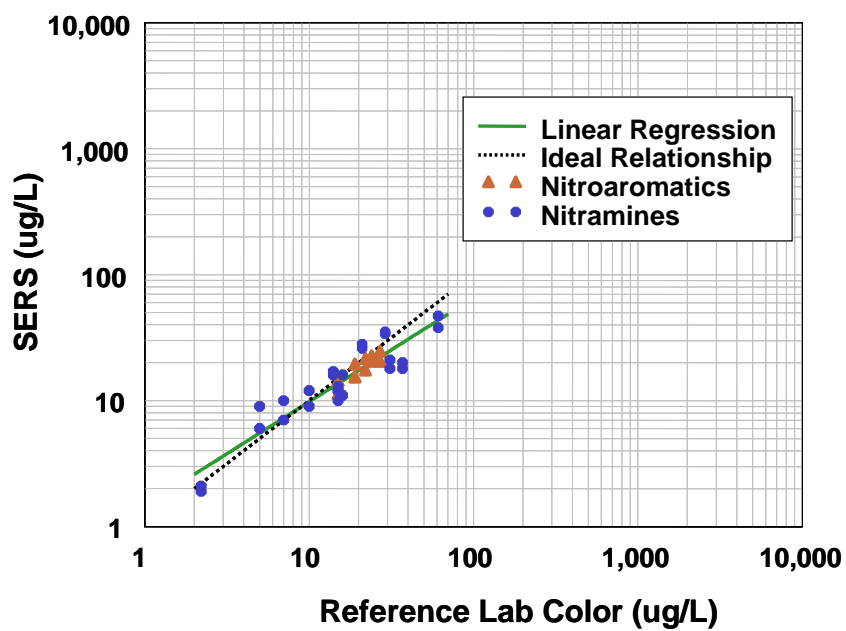


Figure 24. Comparison of SERS and reference laboratory colorimetry results.

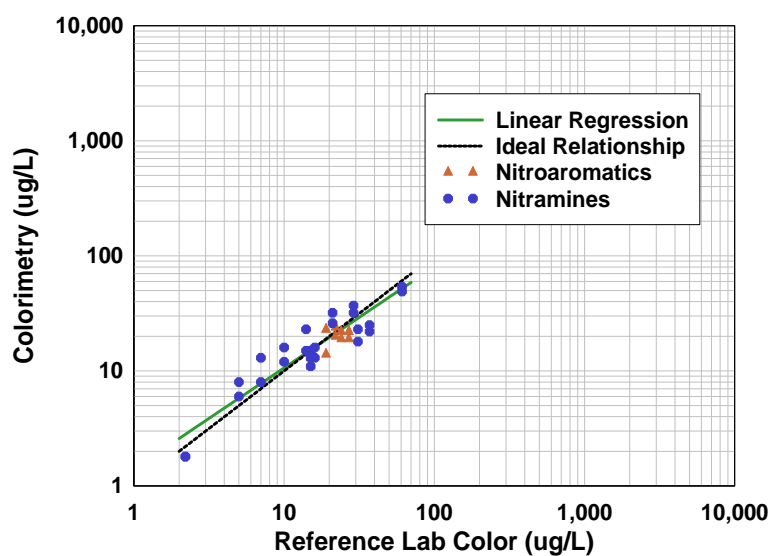


Figure 25. Comparison of colorimetry and reference laboratory colorimetry results.

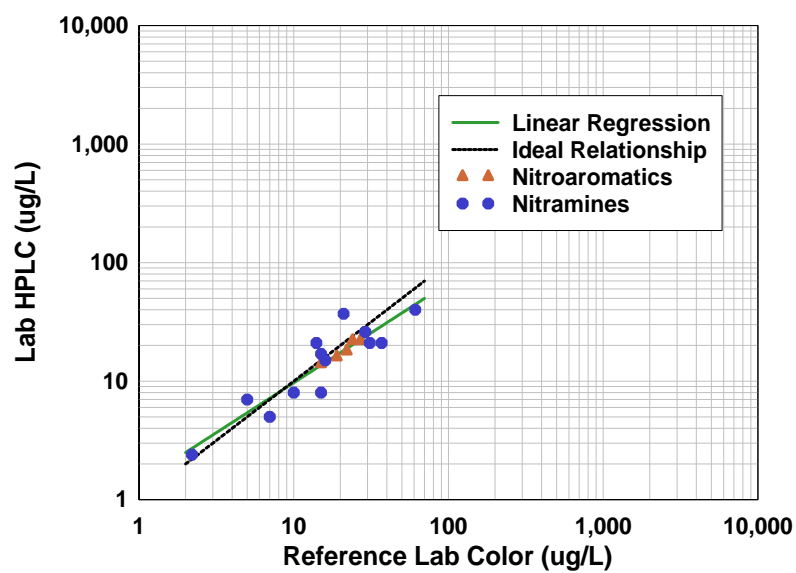


Figure 26. Comparison of HPLC and reference laboratory colorimetry results.

Relative percent differences (RPDs) were also calculated to test the agreement between methods. Table 10 includes mean and median absolute RPDs and net RPDs (average when sign is taken into account) for the same analytical method comparisons summarized in Tables 7 and 9. Ideally, RPDs should be zero. Large absolute RPD values indicate poor agreement between the methods and net RPD values significantly different from zero indicate bias in the data.

The RPD results were consistent with the linear regression and paired statistical results. Single method field and laboratory comparisons as well as SERS vs. colorimetry comparisons yielded the lowest mean absolute RPD values (ca. 15%) and mean net RPD values near zero. Nitramines were again biased high for the colorimetric method relative to both SERS and HPLC, suggesting possible matrix interferences in the water samples at UMCD. Mean absolute RPD values (ca. 25-30%) were higher and mean net RPD values deviated further from zero when SERS and colorimetry were compared against Method 8330 (data sets 3 and 4 in Table 10). The negative mean net RPD values in those data sets suggest some positive bias in the SERS and colorimetry results; however, most of the median net RPD values were close to zero – indicating a nearly equal number of values greater and less than the reference method values and low bias in the data. Comparisons against the reference laboratory HPLC results again yielded the poorest results – mean absolute RPDs were about 45% and both mean and median net RPDs were far from zero. All of the RPDs were negative, indicating that the reference laboratory HPLC results were consistently low relative to the other methods. Lastly, comparisons against the reference laboratory colorimetry results for UMCD samples yielded moderate mean absolute RPD values (ca. 25%) and positive mean and median net RPD values. The positive RPD values indicate that the reference laboratory results were consistently higher than the other methods, although the differences were not large.

Table 11 presents some of the RPDs from the present study along with RPDs reported from other studies as summarized in Reference 22. All of the methods were compared against Method 8330. Results from this study have the difference compared (e.g., FSER-LC) indicated in parenthesis in the Method column. Our methods, including SERS, compare favorably against the other methods, even in the worst case (comparison against the reference laboratory HPLC results).

In summary, the statistical analysis of the demonstration data presented above met our goals set forth at the beginning of this section and supports the following conclusions:

- Field SERS is an analytically acceptable alternative to HPLC or colorimetry for the analysis of explosives in water
- The performance of SERS in the field is comparable to laboratory SERS
- There is no consistent difference in SERS performance for different individual explosive analytes vs. the reference Method 8330
- There is no significant bias in the SERS method (i.e., matrix effects)
- SERS precision is better than interlaboratory reference method results, but considerably poorer (ca. 5-fold higher % RSD and large RPDs in Table 10) than the single laboratory HPLC method for all analytes

Table 10. Relative Percent Difference (RPD) Results

			Absolute RPD		Net RPD	
Difference Compared	Analyte	N	Mean	Median	Mean	Median
LSER-FSER	Combined	51	17.8	13.2	-1.4	6.1
LSER-FSER	RDX	12	19.9	20.0	-1.6	0.0
LSER-FSER	2,4-DNT	19	12.7	10.2	1.1	6.1
LSER-FSER	TNT	14	13.2	11.3	7.6	10.0
LColor-FColor	Combined	32	16.2	13.6	-3.0	-4.6
LColor-FColor	Nitramines	9	24.8	21.7	-5.4	-7.1
LColor-FColor	Nitroaromatics	23	12.9	13.3	-2.0	-3.2
LC-FSER	Combined	57	27.2	15.0	-10.8	1.4
LC-FSER	RDX	13	30.0	27.3	-7.5	0.0
LC-FSER	2,4-DNT	20	18.4	15.7	-4.1	2.4
LC-FSER	TNT	15	34.2	11.7	-19.8	8.3
LC-LSER	Combined	57	26.2	15.4	-11.6	1.4
LC-LSER	RDX	13	21.6	23.5	-5.2	-6.7
LC-LSER	2,4-DNT	20	39.1	26.1	-33.7	-16.9
LC-LSER	TNT	15	34.5	10.0	-28.8	-7.4
LC-LColor	Combined	32	16.5	13.6	-1.9	1.1
LC-LColor	Nitramines	9	25.0	19.0	-14.4	-9.5
LC-LColor	Nitroaromatics	23	13.2	13.4	3.0	5.2
FColor-FSER	Combined	32	15.6	11.8	1.6	4.3
FColor-FSER	Nitramines	9	18.3	18.8	13.1	18.8
FColor-FSER	Nitroaromatics	23	14.6	9.8	-2.9	0.0
LColor-LSER	Combined	32	12.8	9.1	-0.9	-2.1
LColor-LSER	Nitramines	9	14.7	12.5	13.0	12.5
LColor-LSER	Nitroaromatics	23	12.1	9.1	-6.4	-6.0
RLC-FSER	Combined	23	40.4	34.5	-28.1	-19.0
RLC-LSER	Combined	23	45.1	28.8	-31.2	-8.8
RLC-LC	Combined	23	41.7	30.8	-17.8	-21.2
RLC-FColor	Combined	13	42.3	26.6	-29.3	-18.6
RLC-LColor	Combined	13	49.6	31.9	-33.6	-16.7
RColor-FSER	Combined	13	25.5	23.8	10.0	20.0
RColor-LSER	Combined	13	19.5	16.7	11.1	11.1
RColor-LC	Combined	32	27.4	20.0	5.9	18.2
RColor-FColor	Combined	13	30.2	20.8	-2.7	11.5
RColor-LColor	Combined	13	20.6	19.7	1.7	8.3

Table 11. Comparison of RPD Results from Different Studies

Method	Analyte	Mean Abs. RPD	Median Abs. RPD	Mean Net RPD
SERS (FSER-LC)	2,4-DNT	18	16	4
SERS (FSER-LC)	TNT	34	12	20
SERS (FSER-RLC)	Combined	40	34	28
Colorimetry (LColor-LC)	Nitroaromatics	13	13	-3
Colorimetry	Nitroaromatics	66; 58; 44	45; 63; 30	66; 58; 22
Immunoassay	TNT	64; 143	48; 152	58; 143
Flow Immunosensor	TNT	47; 52; 114; 100	47; 38; 147; 89	32; 51; -41; 87
Fiber-Optic Biosensor	TNT	33; 107; 85; 55	25; 116; 74; 52	30; 100; 67; 40
SERS (FSER-LC)	RDX	30	27	8
Colorimetry (LColor-LC)	Nitramines	25	19	14
Colorimetry	Nitramines	33; 21; 31	27; 21; 32	-11; -8; -6
Immunoassay	RDX	53; 67	32; 56	-36; 61
Flow Immunosensor	RDX	26; 30; 78; 76	19; 23; 78; 68	-11; -30; -63; -42
Fiber-Optic Biosensor	RDX	37; 56; 100	33; 40; 104	10; 14; -100

5. Cost Assessment

5.1 Cost Reporting

Our goal in this project was to achieve a SERS analysis cost of less than \$75 per sample for “standard” water samples (i.e., using an analyst). We believe a similar cost target is reasonable for at-line process monitoring because the additional capital equipment costs for process monitoring equipment (sampling and control hardware as well as software) will be offset by lower operator labor costs. For example, at the UMCD GAC plant, annual monitoring costs are approximately \$100K, most of which are labor related. With 20 years or more remediation monitoring expected, there is considerable opportunity for capital expenditure to reduce total costs. In the case of CPT-based analysis, the bulk of the cost is driven by the CPT costs (approximately \$3K per day) and thus analysis costs are of lesser significance. Nevertheless, CPT-based analysis costs are expected to be approximately the same as “standard” SERS analysis costs because the cost of sampling equipment is comparable to other commercial sampling systems and all other cost elements are the same as for “standard” SERS. *In situ* CPT SERS is somewhat more expensive due to additional equipment costs for specialized down-hole equipment and longer fiber optic cables that are expected to be replaced on an annual basis (refer to ancillary equipment in Table 12).

In order to assess the cost per sample as accurately as possible, we tracked the equipment, materials and labor costs during the course of the demonstration. Table 12 summarizes the major cost elements that were tracked. Other cost elements associated with site characterization, such as sampling, mobilization/demobilization and environmental safety training, vary by site and are the same for both SERS and baseline technologies. Therefore, those elements do not significantly impact per sample costs and do not need to be considered in the cost comparison.

Table 12. SERS Cost Tracking

Cost Category	Sub Category	Cost No Preconc.	Cost Preconc.	Cost <i>In Situ</i> CPT
Capital Costs	Capital equipment purchase	\$52,000	\$52,000	\$52,000
	Ancillary equipment purchase	\$0	\$0	\$10,000/yr
Operating Costs	Operator labor/sample (\$60/hr)	\$15	\$20	\$15
	Operator training (1 day)	\$480	\$480	\$480
	Consumables, supplies/sample	\$5	\$14	\$5
	Residual waste handling and disposal	negligible	negligible	negligible

Table 12 considers the difference in productivity and costs between samples requiring preconcentration (e.g., low $\mu\text{g/L}$ detection of nitramines) and those that do not. Normally a \$60/hr (loaded cost) technician can analyze approximately four water samples an hour. Productivity is reduced to three samples an hour if preconcentration is required. For *in situ* CPT- SERS, costs of the basic capital SERS equipment, consumables and labor/sample are approximately the same as for “standard” SERS; however it assumed that the down-hole SERS module (\$6K) and fiber optic cable (\$4K) will have to be replaced once annually due to wear or damage. SERS capital equipment is based on the purchase prices in this study, which are as follows:

- Spectrograph \$15K
- Detector \$15K
- Raman probe \$5K
- Laser \$12K
- Computer \$2K
- Software \$3K

Raman equipment is currently considered specialty instrumentation and is not widely used outside laboratories. Thus, Raman equipment is not available for rental as an alternative to purchase.

The costs for reference laboratory Method 8330 and colorimetry analyses were obtained from SAIC and SCS Engineers. HPLC costs can vary widely (up to several \$K per sample) depending on sample turn-around time, number of analytes reported, etc. To facilitate comparison with SERS and colorimetry, we obtained the costs for routine HPLC analysis reporting just two analytes. Both laboratories reported a lowest cost of \$150 per sample. The laboratory performing colorimetric analysis charged \$80 per sample for two tests - nitramines and nitroaromatics. The cost is \$50 for a single colorimetry test.

5.2 Cost Analysis

The cost of the SERS technology is compared against colorimetry and Method 8330 in Table 13 using the major SERS cost drivers identified in Table 12. The colorimetry cost estimate of \$42 agrees well with commercial prices of \$50 for a single test and previous reports comparing field method costs.^{8,22} The HPLC cost was derived as discussed in Section 5.1. The waste generated by the SERS method is small (see Figure 13) and contributes negligibly to per-sample costs. HPLC generates considerably more waste per sample, which contributes to the higher HPLC costs of \$150 per sample.

Considering only labor and consumables, the basic SERS method costs less than half the cost for a single colorimetric test and is well within our targeted price goal of \$75/test. However, if both nitramines and nitroaromatics are tested (at a total cost of about \$80), basic SERS costs (no preconcentration) drop to just 25% of colorimetry and 15% of HPLC costs. With preconcentration, SERS costs are still less than half the expense of colorimetry for two tests and about 25% of HPLC costs.

Table 13. Comparison of Method Costs

SERS			Colorimetry		HPLC
Consumables	No Prec.	Preconc.	Consumables		
Colloidal Gold	\$1.00	\$3.00	Test Kit (SDI)	\$25.00	
Filter	\$1.00	\$1.00	Alumina Cartridge	\$2.00	
Vial, pipettes, etc.	\$2.00	\$3.00			
Solid Phase Cartridge	n/a	\$6.00			
Standards	\$1.00	\$1.00			
Subtotal	\$5.00	\$14.00	Subtotal	\$27.00	
Labor			Labor		
Technician (\$60/hr)	\$15.00	\$20.00	Technician (\$60/hr)	\$15.00	
Total	\$20.00	\$34.00	Total	\$42.00	\$150.00
Equipment			Equipment		
Raman Spectrometer	\$52,000	\$52,000	Spectrophotometer	\$2,000	
Waste generated	1 mL	3 mL	Waste generated	25 mL	>> 25 mL

SERS life cycle costs are dominated by capital equipment expenses that can be reasonably amortized over a five-year period. A useful means to compare the costs of different methods that include capital equipment is to calculate the break-even point. For “standard” SERS, the break-even point (i.e., the number of samples that must be analyzed to pay off the equipment and immediately realize the lower per-sample costs identified in Table 13) against Method 8330 is 400 samples without preconcentration and 450 samples with preconcentration. Assuming both nitramines and nitroaromatics are analyzed in each sample, the break-even point against colorimetry is about 850 samples without preconcentration and about 1100 samples with

preconcentration. These numbers are small when considered over a 5-year period (only 100-200 or so samples per year). Viewed another way, the least favorable break-even point (against colorimetry with SERS preconcentration) is reached in a total cost of \$89K which is less than one year's monitoring cost at the UMCD GAC plant. Clearly, SERS is a potentially cost-saving alternative to both HPLC and colorimetry.

In situ CPT SERS break-even points (without preconcentration only) are approximately double the "standard" SERS break-even points due to annual down-hole equipment replacement costs. Costs can be recouped faster, however, because a premium of up to \$500-\$1000/day can be charged for specialized CPT work. It is also important to note, as discussed earlier in this report, that most CPT SERS work is expected to be performed entirely up-hole because of the advantages of lower cost, less complexity, and greater opportunity for sample preparation (e.g., preconcentration).

6. Implementation Issues

6.1 Environmental Checklist

At ALAAP, there were no regulations or permitting requirements that applied to the demonstration, which was conducted under the auspices of the existing site Health & Safety Plan. At UMCD, each CPT hole required a closure permit. Permitting was handled by the Army Corps of Engineers geologist on site.

6.2 Other Regulatory Issues

We are in regular contact with EPA regulators from several EPA regions (e.g., 1, 3, 4, and 10) and have begun to disseminate information to them and EPA Headquarters (OSW) personnel directly regarding the SERS technology. As the first step toward receiving regulatory acceptance of the method, we are pursuing a six-state MOU. The SERS method should also be suitable as a Tier 1 single laboratory, single matrix EPA method as described in the agency's *Guide to Method Flexibility and Approval of EPA Water Methods* (Office of Water) and/or as an approved OSW method. Round-robin, inter-laboratory validation of the method will be required for approval.

The SERS technology is largely transparent to the public, except to the extent it lowers monitoring costs which the citizenry strongly supports. There is no call for direct public participation in the technology.

6.3 End-User Issues

End users for the SERS technology are on-site environmental services companies currently performing sample collection and field analyses. Discussions with SAIC, IT and SCS Engineers contractor personnel, as well as U.S. Army Corps of Engineers oversight personnel, indicate a ready willingness to implement new field screening technologies that overcome the interference

problems of existing field methods as long as low $\mu\text{g/L}$ level sensitivity is available and costs are comparable to or less than colorimetric, immunoassay and other field techniques (see the discussion in Sections 1.1 and 1.4). The contractors are ultimately interested in purchasing equipment, supplies and being trained to perform the analyses, or contracting for SERS analytical services. As part of our growing environmental services business, including CPT, we envision initial entry into the market by providing on-site SERS analysis ourselves. Indeed, we have already used the method at two, private former explosive manufacturing plants in Canada and the U.S. over the past year. Current plans are to perform follow-on CPT SERS service work to better characterize the UMCD RDX groundwater plume and locate additional sentinel wells. We are also working with SCS Engineers and the Army Corps to develop a plan for manufacturing and implementing an unattended process monitor for the UMCD GAC Plant. Through our existing sales network or distributors, ARA plans to offer the SERS equipment, supplies, and training for commercial sale. We foresee no major impediments to meeting this goal.

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8. Points of Contact

Table 14. Points of Contact

Point of Contact	Organization	Phone/Fax/email	Role in Project
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R. Doug Webb	USACE 109 St. Josephs Street Mobile, AL 36602	(334) 690-3476 (334) 690-2030 ronald.d.webb@sam.usace.army.mil	ALAAP Govt Demo Host
Andrejs Dimbirs, P.G.	USACE P.O. Box 3755 Seattle, WA 98124	(206) 764-6921 (206) 764-3706 Fax andrejs.p.dimbirs@usace.army.mil	UMCD Govt Demo Host

Submitted by:

<signed> John W. Haas III

3 April 2006

Dr. John Haas, ARA
Principal Investigator

Date

APPENDIX A

U.S. EPA SW-846 ANALYTICAL METHOD 8330

METHOD 8330

NITROAROMATICS AND NITRAMINES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

1.0 SCOPE AND APPLICATION

1.1 Method 8330 is intended for the trace analysis of explosives residues by high performance liquid chromatography using a UV detector. This method is used to determine the concentration of the following compounds in a water, soil, or sediment matrix:

Compound	Abbreviation	CAS No ^a
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	2691-41-0
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4
1,3,5-Trinitrobenzene	1,3,5-TNB	99-35-4
1,3-Dinitrobenzene	1,3-DNB	99-65-0
Methyl-2,4,6-trinitrophenylnitramine	Tetryl	479-45-8
Nitrobenzene	NB	98-95-3
2,4,6-Trinitrotoluene	2,4,6-TNT	118-96-7
4-Amino-2,6-dinitrotoluene	4-Am-DNT	1946-51-0
2-Amino-4, 6-dinitrotoluene	2-Am-DNT	355-72-78-2
2,4-Dinitrotoluene	2,4-DNT	121-14-2
2,6-Dinitrotoluene	2,6-DNT	606-20-2
2-Nitrotoluene	2-NT	88-72-2
3-Nitrotoluene	3-NT	99-08-1
4-Nitrotoluene	4-NT	99-99-0

a Chemical Abstracts Service Registry number

1.2 Method 8330 provides a salting-out extraction procedure for low concentration (parts per trillion, or nanograms per liter) of explosives residues in surface or ground water. Direct injection of diluted and filtered water samples can be used for water samples of higher concentration (See Table 1).

1.3 All of these compounds are either used in the manufacture of explosives or are the degradation products of compounds used for that purpose. When making stock solutions for calibration, treat each explosive compound with caution. See NOTE in Sec. 5.3.1 and Sec. 11 on Safety.

1.4 The estimated quantitation limits (EQLs) of target analytes determined by Method 8330 in water and soil are presented in Table 1.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC, skilled in the interpretation of

chromatograms, and experienced in handling explosive materials. (See Sec. 11.0 on SAFETY.) Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Method 8330 provides high performance liquid chromatographic (HPLC) conditions for the detection of ppb levels of certain explosives residues in water, soil and sediment matrix. Prior to use of this method, appropriate sample preparation techniques must be used.

2.2 Low-Level Salting-out Method With No Evaporation: Aqueous samples of low concentration are extracted by a salting-out extraction procedure with acetonitrile and sodium chloride. The small volume of acetonitrile that remains undissolved above the salt water is drawn off and transferred to a smaller volumetric flask. It is back-extracted by vigorous stirring with a specific volume of salt water. After equilibration, the phases are allowed to separate and the small volume of acetonitrile residing in the narrow neck of the volumetric flask is removed using a Pasteur pipet. The concentrated extract is diluted 1:1 with reagent grade water. An aliquot is separated on a C-18 reverse phase column, determined at 254 nm, and confirmed on a CN reverse phase column.

2.3 High-level Direct Injection Method: Aqueous samples of higher concentration can be diluted 1/1 (v/v) with methanol or acetonitrile, filtered, separated on a C-18 reverse phase column, determine at 254 nm, and confirmed on a CN reverse phase column. If HMX is an important target analyte, methanol is preferred.

2.4 Soil and sediment samples are extracted using acetonitrile in an ultrasonic bath, filtered and chromatographed as in Sec. 2.3.

3.0 INTERFERENCES

3.1 Solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts and/or elevated baselines, causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free from interferences.

3.2 2,4-DNT and 2,6-DNT elute at similar retention times (retention time difference of 0.2 minutes). A large concentration of one isomer may mask the response of the other isomer. If it is not apparent that both isomers are present (or are not detected), an isomeric mixture should be reported.

3.3 Tetryl decomposes rapidly in methanol/water solutions, as well as with heat. All aqueous samples expected to contain tetryl should be diluted with acetonitrile prior to filtration and acidified to pH <3. All samples expected to contain tetryl should not be exposed to temperatures above room temperature.

3.4 Degradation products of tetryl appear as a shoulder on the 2,4,6-TNT peak. Peak heights rather than peak areas should be used when tetryl is present in concentrations that are significant relative to the concentration of 2,4,6-TNT.

4.0 APPARATUS AND MATERIALS

4.1 HPLC system

4.1.1 HPLC - equipped with a pump capable of achieving 4000 psi, a 100 μ l loop injector and a 254 nm UV detector (Perkin Elmer Series 3, or equivalent). For the low concentration option, the detector must be capable of a stable baseline at 0.001 absorbance units full scale.

4.1.2 Recommended Columns:

4.1.2.1 Primary column: C-18 Reverse phase HPLC column, 25 cm x 4.6 mm (5 μ m), (Supelco LC-18, or equivalent).

4.1.2.2 Secondary column: CN Reverse phase HPLC column, 25 cm x 4.6 mm (5 μ m), (Supelco LC-CN, or equivalent).

4.1.3 Strip chart recorder.

4.1.4 Digital integrator (optional).

4.1.5 Autosampler (optional).

4.2 Other Equipment

4.2.1 Temperature controlled ultrasonic bath.

4.2.2 Vortex mixer.

4.2.3 Balance, \pm 0.0001 g.

4.2.4 Magnetic stirrer with stirring pellets.

4.2.5 Water bath - Heated, with concentric ring cover, capable of temperature control (\pm 5°C). The bath should be used in a hood.

4.2.6 Oven - Forced air, without heating.

4.3 Materials

4.3.1 High pressure injection syringe - 500 μ L, (Hamilton liquid syringe or equivalent).

4.3.2 Disposable cartridge filters - 0.45 μ m Teflon filter.

4.3.3 Pipets - Class A, glass, Appropriate sizes.

4.3.4 Pasteur pipets.

4.3.5 Scintillation Vials - 20 mL, glass.

4.3.6 Vials - 15 mL, glass, Teflon-lined cap.

4.3.7 Vials- 40 mL, glass, Teflon-lined cap.

4.3.8 Disposable syringes - Plastipak, 3 mL and 10 mL or equivalent.

4.3.9 Volumetric flasks - Appropriate sizes with ground glass stoppers, Class A.

NOTE: The 100 mL and 1 L volumetric flasks used for magnetic stirrer extraction must be round.

4.3.10 Vacuum desiccator - Glass.

4.3.11 Mortar and pestle - Steel.

4.3.12 Sieve - 30 mesh.

4.3.13 Graduated cylinders - Appropriate sizes.

4.4 Preparation of Materials

4.4.1 Prepare all materials to be used as described in Chapter 4 for semivolatile organics.

5.0 REAGENTS

5.1 Reagent grade inorganic chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lowering the accuracy of the determination.

5.1.1 Acetonitrile, CH_3CN - HPLC grade.

5.1.2 Methanol, CH_3OH - HPLC grade.

5.1.3 Calcium chloride, CaCl_2 - Reagent grade. Prepare an aqueous solution of 5 g/L.

5.1.4 Sodium chloride, NaCl , shipped in glass bottles - reagent grade.

5.2 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Stock Standard Solutions

5.3.1 Dry each solid analyte standard to constant weight in a vacuum desiccator in the dark. Place about 0.100 g (weighed to 0.0001 g) of a single analyte into a 100 mL volumetric flask and dilute to volume with

acetonitrile. Invert flask several times until dissolved. Store in refrigerator at 4°C in the dark. Calculate the concentration of the stock solution from the actual weight used (nominal concentration = 1,000 mg/L). Stock solutions may be used for up to one year.

NOTE: The HMX, RDX, Tetryl, and 2,4,6-TNT are explosives and the neat material should be handled carefully. See SAFETY in Sec. 11 for guidance. HMX, RDX, and Tetryl reference materials are shipped under water. Drying at ambient temperature requires several days. DO NOT DRY AT HEATED TEMPERATURES!

5.4 Intermediate Standards Solutions

5.4.1 If both 2,4-DNT and 2,6-DNT are to be determined, prepare two separate intermediate stock solutions containing (1) HMX, RDX, 1,3,5-TNB, 1,3-DNB, NB, 2,4,6-TNT, and 2,4-DNT and (2) Tetryl, 2,6-DNT, 2-NT, 3-NT, and 4-NT. Intermediate stock standard solutions should be prepared at 1,000 µg/L, in acetonitrile when analyzing soil samples, and in methanol when analyzing aqueous samples.

5.4.2 Dilute the two concentrated intermediate stock solutions, with the appropriate solvent, to prepare intermediate standard solutions that cover the range of 2.5 - 1,000 µg/L. These solutions should be refrigerated on preparation, and may be used for 30 days.

5.4.3 For the low-level method, the analyst must conduct a detection limit study and devise dilution series appropriate to the desired range. Standards for the low level method must be prepared immediately prior to use.

5.5 Working standards

5.5.1 Calibration standards at a minimum of five concentration levels should be prepared through dilution of the intermediate standards solutions by 50% (v/v) with 5 g/L calcium chloride solution (Sec. 5.1.3). These solutions must be refrigerated and stored in the dark, and prepared fresh on the day of calibration.

5.6 Surrogate Spiking Solution

5.6.1 The analyst should monitor the performance of the extraction and analytical system as well as the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard and reagent water blank with one or two surrogates (e.g., analytes not expected to be present in the sample).

5.7 Matrix Spiking Solutions

5.7.1 Prepare matrix spiking solutions in methanol such that the concentration in the sample is five times the Estimated Quantitation Limit (Table 1). All target analytes should be included.

5.8 HPLC Mobile Phase

5.8.1 To prepare 1 liter of mobile phase, add 500 mL of methanol to 500 mL of organic-free reagent water.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 Follow conventional sampling and sample handling procedures as specified for semivolatile organics in Chapter Four.

6.2 Samples and sample extracts must be stored in the dark at 4°C. Holding times are the same as for semivolatile organics.

7.0 PROCEDURE

7.1 Sample Preparation

7.1.1 Aqueous Samples: It is highly recommended that process waste samples be screened with the high-level method to determine if the low level method (1-50 µg/L) is required. Most groundwater samples will fall into the low level method.

7.1.1.1 Low-Level Method (salting-out extraction)

7.1.1.1.1 Add 251.3 g of sodium chloride to a 1 L volumetric flask (round). Measure out 770 mL of a water sample (using a 1 L graduated cylinder) and transfer it to the volumetric flask containing the salt. Add a stir bar and mix the contents at maximum speed on a magnetic stirrer until the salt is completely dissolved.

7.1.1.1.2 Add 164 mL of acetonitrile (measured with a 250 mL graduated cylinder) while the solution is being stirred and stir for an additional 15 minutes. Turn off the stirrer and allow the phases to separate for 10 minutes.

7.1.1.1.3 Remove the acetonitrile (upper) layer (about 8 mL) with a Pasteur pipet and transfer it to a 100 mL volumetric flask (round). Add 10 mL of fresh acetonitrile to the water sample in the 1 L flask. Again stir the contents of the flask for 15 minutes followed by 10 minutes for phase separation. Combine the second acetonitrile portion with the initial extract. The inclusion of a few drops of salt water at this point is unimportant.

7.1.1.1.4 Add 84 mL of salt water (325 g NaCl per 1000 mL of reagent water) to the acetonitrile extract in the 100 mL volumetric flask. Add a stir bar and stir the contents on a magnetic stirrer for 15 minutes, followed by 10 minutes for phase separation. Carefully transfer the acetonitrile phase

to a 10 mL graduated cylinder using a Pasteur pipet. At this stage, the amount of water transferred with the acetonitrile must be minimized. The water contains a high concentration of NaCl that produces a large peak at the beginning of the chromatogram, where it could interfere with the HMX determination.

7.1.1.1.5 Add an additional 1.0 mL of acetonitrile to the 100 mL volumetric flask. Again stir the contents of the flask for 15 minutes, followed by 10 minutes for phase separation. Combine the second acetonitrile portion with the initial extract in the 10 mL graduated cylinder (transfer to a 25 mL graduated cylinder if the volume exceeds 5 mL). Record the total volume of acetonitrile extract to the nearest 0.1 mL. (Use this as the volume of total extract [V_t] in the calculation of concentration after converting to μL). The resulting extract, about 5 - 6 mL, is then diluted 1:1 with organic-free reagent water (with pH <3 if tetryl is a suspected analyte) prior to analysis.

7.1.1.1.6 If the diluted extract is turbid, filter it through a 0.45 - μm Teflon filter using a disposable syringe. Discard the first 0.5 mL of filtrate, and retain the remainder in a Teflon-capped vial for RP-HPLC analysis as in Sec. 7.4.

7.1.1.2 High-Level Method

7.1.1.2.1 Sample filtration: Place a 5 mL aliquot of each water sample in a scintillation vial, add 5 mL of acetonitrile, shake thoroughly, and filter through a 0.45- μm Teflon filter using a disposable syringe. Discard the first 3 mL of filtrate, and retain the remainder in a Teflon-capped vial for RP-HPLC analysis as in Sec. 7.4. HMX quantitation can be improved with the use of methanol rather than acetonitrile for dilution before filtration.

7.1.2 Soil and Sediment Samples

7.1.2.1 Sample homogenization: Dry soil samples in air at room temperature or colder to a constant weight, being careful not to expose the samples to direct sunlight. Grind and homogenize the dried sample thoroughly in an acetonitrile-rinsed mortar to pass a 30 mesh sieve.

NOTE: Soil samples should be screened by Method 8515 prior to grinding in a mortar and pestle (See Safety Sec. 11.2).

7.1.2.2 Sample extraction

7.1.2.2.1 Place a 2.0 g subsample of each soil sample in a 15 mL glass vial. Add 10.0 mL of acetonitrile, cap with

Teflon-lined cap, vortex swirl for one minute, and place in a cooled ultrasonic bath for 18 hours.

7.1.2.2.2 After sonication, allow sample to settle for 30 minutes. Remove 5.0 mL of supernatant, and combine with 5.0 mL of calcium chloride solution (Sec. 5.1.3) in a 20 mL vial. Shake, and let stand for 15 minutes.

7.1.2.2.3 Place supernatant in a disposable syringe and filter through a 0.45- μ m Teflon filter. Discard first 3 mL and retain remainder in a Teflon-capped vial for RP-HPLC analysis as in Sec. 7.4.

7.2 Chromatographic Conditions (Recommended)

Primary Column: C-18 reverse phase HPLC column, 25-cm x 4.6-mm, 5 μ m, (Supelco LC-18 or equivalent).

Secondary Column: CN reverse phase HPLC column, 25-cm x 4.6-mm, 5 μ m, (Supelco LC-CN or equivalent).

Mobile Phase: 50/50 (v/v) methanol/organic-free reagent water.

Flow Rate: 1.5 mL/min

Injection volume: 100- μ L

UV Detector: 254 nm

7.3 Calibration of HPLC

7.3.1 All electronic equipment is allowed to warm up for 30 minutes. During this period, at least 15 void volumes of mobile phase are passed through the column (approximately 20 min at 1.5 mL/min) and continued until the baseline is level at the UV detector's greatest sensitivity.

7.3.2 Initial Calibration. Injections of each calibration standard over the concentration range of interest are made sequentially into the HPLC in random order. Peak heights or peak areas are obtained for each analyte. Experience indicates that a linear calibration curve with zero intercept is appropriate for each analyte. Therefore, a response factor for each analyte can be taken as the slope of the best-fit regression line.

7.3.3 Daily Calibration. Analyze midpoint calibration standards, at a minimum, at the beginning of the day, singly at the midpoint of the run, and singly after the last sample of the day (assuming a sample group of 10 samples or less). Obtain the response factor for each analyte from the mean peak heights or peak areas and compare it with the response factor obtained for the initial calibration. The mean response factor for the

daily calibration must agree within $\pm 15\%$ of the response factor of the initial calibration. The same criteria is required for subsequent standard responses compared to the mean response of the triplicate standards beginning the day. If this criterion is not met, a new initial calibration must be obtained.

7.4 HPLC Analysis

7.4.1 Analyze the samples using the chromatographic conditions given in Sec. 7.2. All positive measurements observed on the C-18 column must be confirmed by injection onto the CN column.

7.4.2 Follow Sec. 7.0 in Method 8000 for instructions on the analysis sequence, appropriate dilutions, establishing daily retention time windows, and identification criteria. Include a mid-level standard after each group of 10 samples in the analysis sequence. If column temperature control is not employed, special care must be taken to ensure that temperature shifts do not cause peak misidentification.

7.4.3 Table 2 summarizes the estimated retention times on both C-18 and CN columns for a number of analytes analyzable using this method. An example of the separation achieved by Column 1 is shown in Figure 1.

7.4.4 Record the resulting peak sizes in peak heights or area units. The use of peak heights is recommended to improve reproducibility of low level samples.

7.4.5 Calculation of concentration is covered in Sec. 7.0 of Method 8000.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One for specific quality control procedures. Quality control to validate sample extraction is covered in Method 3500.

8.2 Quality control required to validate the HPLC system operation is found in Method 8000, Sec. 8.0.

8.3 Prior to preparation of stock solutions, acetonitrile, methanol, and water blanks should be run to determine possible interferences with analyte peaks. If the acetonitrile, methanol, or water blanks show contamination, a different batch should be used.

9.0 METHOD PERFORMANCE

9.1 Table 3 presents the single laboratory precision based on data from the analysis of blind duplicates of four spiked soil samples and four field contaminated samples analyzed by seven laboratories.

9.2 Table 4 presents the multilaboratory error based on data from the analysis of blind duplicates of four spiked soil samples and four field contaminated samples analyzed by seven laboratories.

9.3 Table 5 presents the multilaboratory variance of the high concentration method for water based on data from nine laboratories.

9.4 Table 6 presents multilaboratory recovery data from the analysis of spiked soil samples by seven laboratories.

9.5 Table 7 presents a comparison of method accuracy for soil and aqueous samples (high concentration method).

9.6 Table 8 contains precision and accuracy data for the salting-out extraction method.

10.0 REFERENCES

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11.0 SAFETY

11.1 Standard precautionary measures used for handling other organic compounds should be sufficient for the safe handling of the analytes targeted by Method 8330. The only extra caution that should be taken is when handling the analytical standard neat material for the explosives themselves and in rare cases where soil or waste samples are highly contaminated with the explosives. Follow the note for drying the neat materials at ambient temperatures.

11.2 It is advisable to screen soil or waste samples using Method 8515 to determine whether high concentrations of explosives are present. Soil samples as high as 2% 2,4,6-TNT have been safely ground. Samples containing higher concentrations should not be ground in the mortar and pestle. Method 8515 is for 2,4,6-TNT, however, the other nitroaromatics will also cause a color to be developed and provide a rough estimation of their concentrations. 2,4,6-TNT is the analyte most often detected in high concentrations in soil samples. Visual observation of a soil sample is also important when the sample is taken from a site expected to contain explosives. Lumps of material that have a chemical appearance should be suspect and not ground. Explosives are generally a very finely ground grayish-white material.

TABLE 1
ESTIMATED QUANTITATION LIMITS

Compounds	Water ($\mu\text{g/L}$)		Soil (mg/kg)
	Low-Level	High-Level	
HMX	-	13.0	2.2
RDX	0.84	14.0	1.0
1,3,5-TNB	0.26	7.3	0.25
1,3-DNB	0.11	4.0	0.25
Tetryl	-	4.0	0.65
NB	-	6.4	0.26
2,4,6-TNT	0.11	6.9	0.25
4-Am-DNT	0.060	-	-
2-Am-DNT	0.035	-	-
2,6-DNT	0.31	9.4	0.26
2,4-DNT	0.020	5.7	0.25
2-NT	-	12.0	0.25
4-NT	-	8.5	0.25
3-NT	-	7.9	0.25

TABLE 2
RETENTION TIMES AND CAPACITY FACTORS ON LC-18 AND LC-CN COLUMNS

Compound	Retention time (min)		Capacity factor (k)*	
	LC-18	LC-CN	LC-18	LC-CN
HMX	2.44	8.35	0.49	2.52
RDX	3.73	6.15	1.27	1.59
1,3,5-TNB	5.11	4.05	2.12	0.71
1,3-DNB	6.16	4.18	2.76	0.76
Tetryl	6.93	7.36	3.23	2.11
NB	7.23	3.81	3.41	0.61
2,4,6-TNT	8.42	5.00	4.13	1.11
4-Am-DNT	8.88	5.10	4.41	1.15
2-Am-DNT	9.12	5.65	4.56	1.38
2,6-DNT	9.82	4.61	4.99	0.95
2,4-DNT	10.05	4.87	5.13	1.05
2-NT	12.26	4.37	6.48	0.84
4-NT	13.26	4.41	7.09	0.86
3-NT	14.23	4.45	7.68	0.88

* Capacity factors are based on an unretained peak for nitrate at 1.71 min on LC-18 and at 2.00 min on LC-CN.

TABLE 3
SINGLE LABORATORY PRECISION OF METHOD FOR SOIL SAMPLES

	Spiked Soils			Field-Contaminated Soils		
	Mean Conc. (mg/kg)	SD	%RSD	Mean Conc. (mg/kg)	SD	%RSD
HMX	46	1.7	3.7	14	1.8	12.8
				153	21.6	14.1
RDX	60	1.4	2.3	104	12	11.5
				877	29.6	3.4
1,3,5-TNB	8.6	0.4	4.6	2.8	0.2	7.1
	46	1.9	4.1	72	6.0	8.3
1,3-DNB	3.5	0.14	4.0	1.1	0.11	9.8
Tetryl	17	3.1	17.9	2.3	0.41	18.0
2,4,6-TNT	40	1.4	3.5	7.0	0.61	9.0
				669	55	8.2
2,4-DNT	5.0	0.17	3.4	1.0	0.44	42.3

TABLE 4
MULTILABORATORY ERROR OF METHOD FOR SOIL SAMPLES

	Spiked Soils			Field-Contaminated Soils		
	Mean Conc. (mg/kg)	SD	%RSD	Mean Conc. (mg/kg)	SD	%RSD
HMX	46	2.6	5.7	14	3.7	26.0
				153	37.3	24.0
RDX	60	2.6	4.4	104	17.4	17.0
				877	67.3	7.7
1,3,5-TNB	8.6	0.61	7.1	2.8	0.23	8.2
	46	2.97	6.5	72	8.8	12.2
1,3-DNB	3.5	0.24	6.9	1.1	0.16	14.5
Tetryl	17	5.22	30.7	2.3	0.49	21.3
2,4,6-TNT	40	1.88	4.7	7.0	1.27	18.0
				669	63.4	9.5
2,4-DNT	5.0	0.22	4.4	1.0	0.74	74.0

TABLE 5
MULTILABORATORY VARIANCE OF METHOD FOR WATER SAMPLES^a

Compounds	Mean Conc. (µg/L)	SD	%RSD
HMX	203	14.8	7.3
RDX	274	20.8	7.6
2,4-DNT	107	7.7	7.2
2,4,6-TNT	107	11.1	10.4

^a Nine Laboratories

TABLE 6
MULTILABORATORY RECOVERY DATA FOR SPIKED SOIL SAMPLES

Laboratory	Concentration (µg/g)						
	HMX	RDX	1,3,5-TNB	1,3-DNB	Tetryl	2,4,6-TNT	2,4-DNT
1	44.97	48.78	48.99	49.94	32.48	49.73	51.05
3	50.25	48.50	45.85	45.96	47.91	46.25	48.37
4	42.40	44.00	43.40	49.50	31.60	53.50	50.90
5	46.50	48.40	46.90	48.80	32.10	55.80	49.60
6	56.20	55.00	41.60	46.30	13.20	56.80	45.70
7	41.50	41.50	38.00	44.50	2.60	36.00	43.50
8	52.70	52.20	48.00	48.30	44.80	51.30	49.10
True Conc	50.35	50.20	50.15	50.05	50.35	50.65	50.05
Mean	47.79	48.34	44.68	47.67	29.24	49.91	48.32
Std Dev	5.46	4.57	3.91	2.09	16.24	7.11	2.78
% RSD	11.42	9.45	8.75	4.39	55.53	14.26	5.76
% Diff*	5.08	3.71	10.91	4.76	41.93	1.46	3.46
Mean % Recovery	95	96	89	95	58	98	96

* Between true value and mean determined value.

TABLE 7
COMPARISON OF METHOD ACCURACY FOR SOIL AND AQUEOUS SAMPLES
(HIGH CONCENTRATION METHOD)

Analyte	Recovery (%)	
	Soil Method*	Aqueous Method**
2,4-DNT	96.0	98.6
2,4,6-TNT	96.8	94.4
RDX	96.8	99.6
HMX	95.4	95.5

* Taken from Bauer et al. (1989), Reference 1.

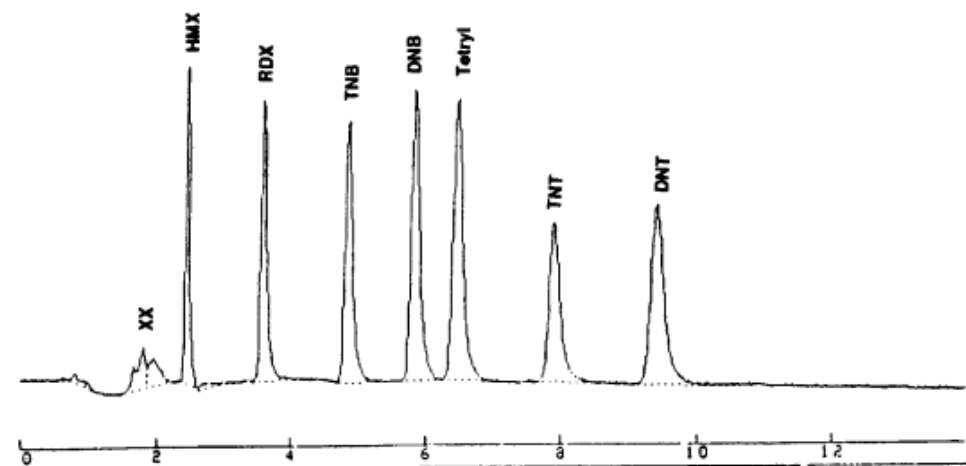
** Taken from Jenkins et al. (1984), Reference 3.

TABLE 8
PRECISION AND ACCURACY DATA FOR THE SALTING-OUT EXTRACTION METHOD

Analyte	No. of Samples ¹	Precision (% RSD)	Ave. Recovery (%)	Conc. Range (µg/L)
HMX	20	10.5	106	0-1.14
RDX	20	8.7	106	0-1.04
1,3,5-TNB	20	7.6	119	0-0.82
1,3-DNB	20	6.6	102	0-1.04
Tetryl	20	16.4	93	0-0.93
2,4,6-TNT	20	7.6	105	0-0.98
2-Am-DNT	20	9.1	102	0-1.04
2,4-DNT	20	5.8	101	0-1.01
1,2-NT	20	9.1	102	0-1.07
1,4-NT	20	18.1	96	0-1.06
1,3-NT	20	12.4	97	0-1.23

¹Reagent water

**EXPLOSIVES ON A
C18 COLUMN**



**EXPLOSIVES ON A
CN COLUMN**

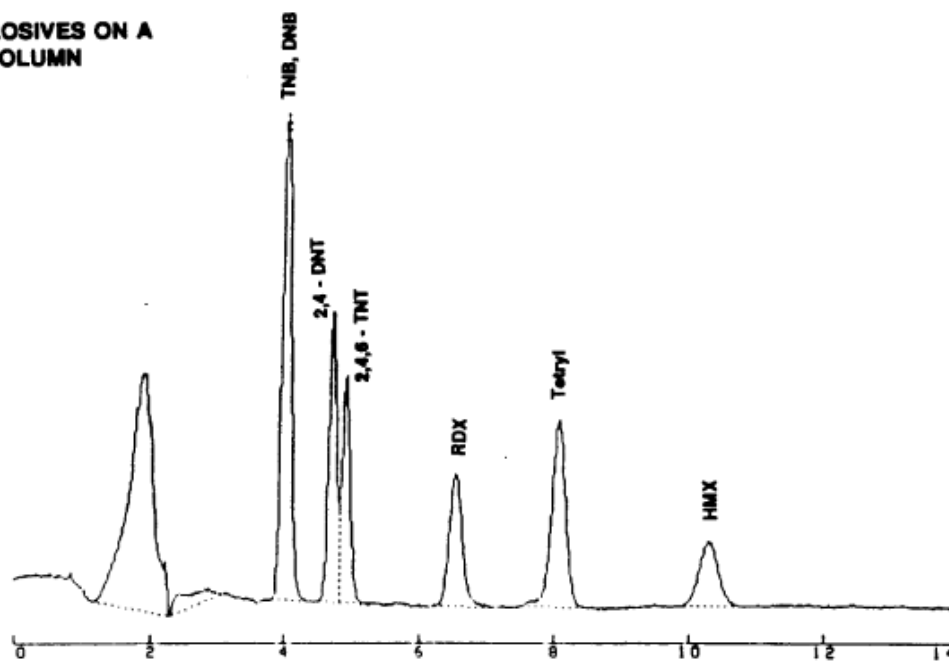
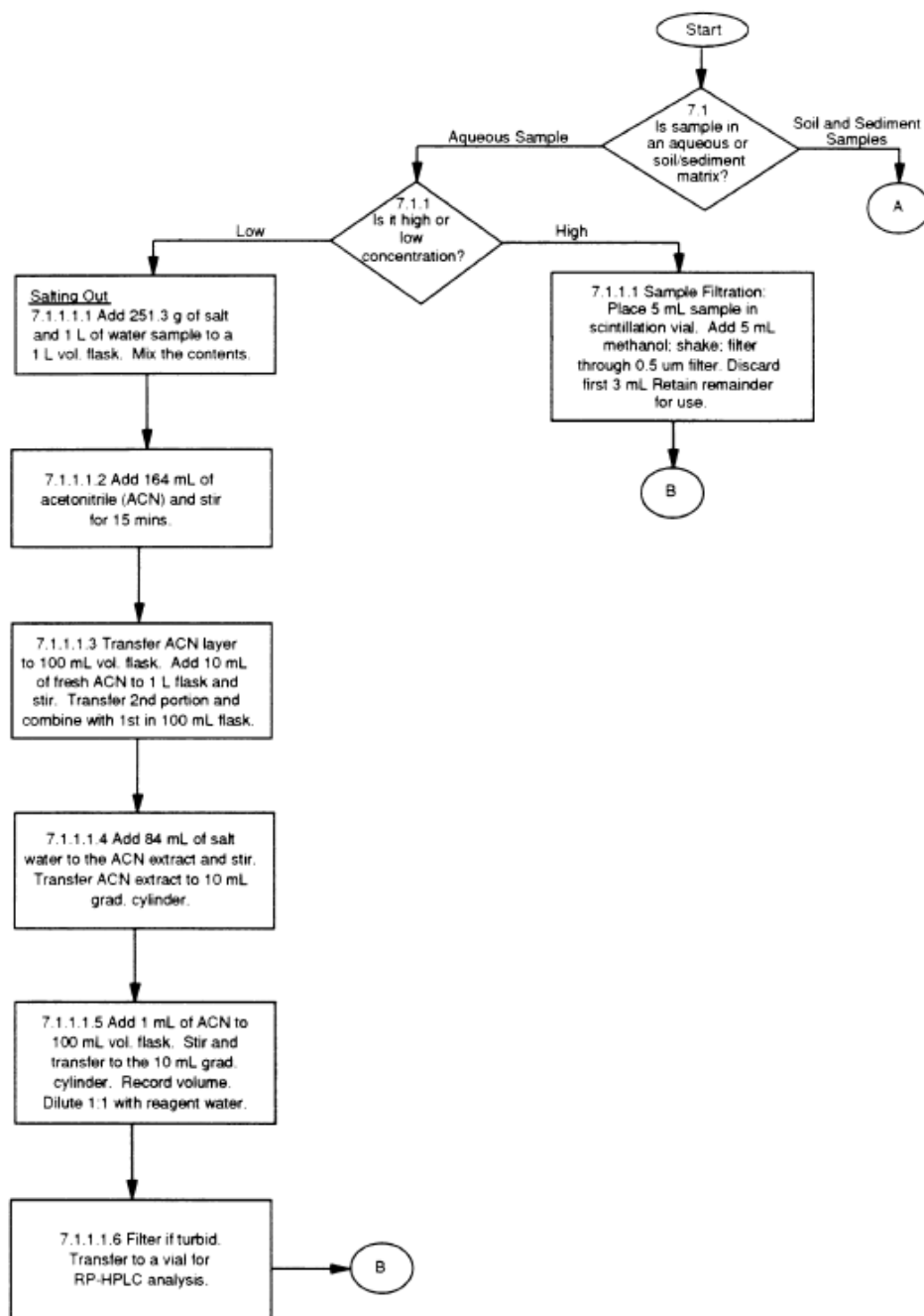
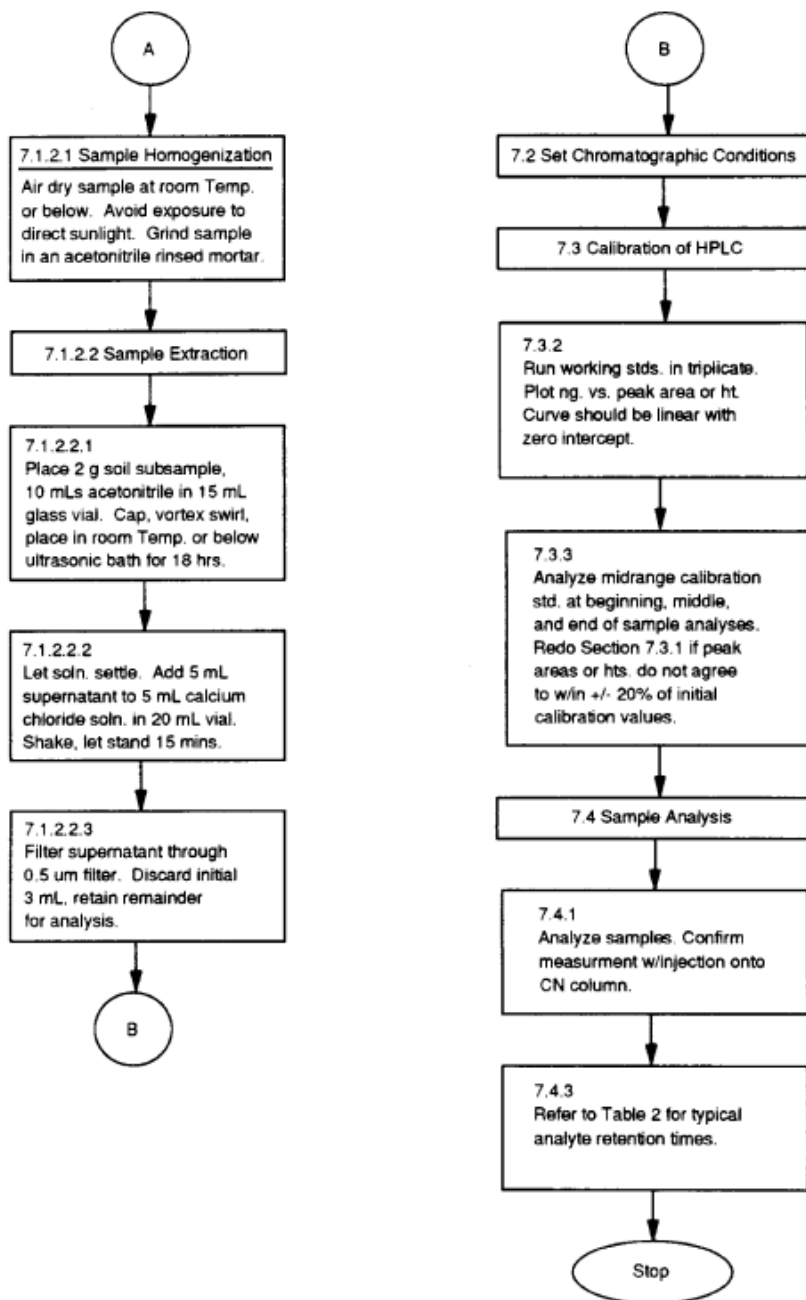


FIGURE 1
CHROMATOGRAMS FOR COLUMNS DESCRIBED IN Sec. 4.1.2.
COURTESY OF U.S. ARMY CORPS OF ENGINEERS, OMAHA, NE.

METHOD 8330
NITROAROMATICS AND NITRAMINES BY HIGH
PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)



METHOD 8330
(continued)



APPENDIX B

QUALITY ASSURANCE PROJECT PLAN

B.1 Purpose and Scope of the Plan

This Quality Assurance Project Plan (QAPP) has been prepared as a guide to ensure that data of sufficient quality are collected to support the objectives of the demonstration as outlined in the Demonstration Plan. The QAPP defines the policy, organization, functional activities, and quality assurance (QA) and quality control (QC) protocols used to help meet the stated demonstrated objectives. Descriptions of quality-related procedures are included in this document and adherence to them should generate data that are scientifically sound and sufficient to derive valid conclusions for the project.

B.2 Quality Assurance Responsibilities

All project personnel are individually responsible for understanding and adhering to the quality procedures described in this document. Ultimate QA responsibility lies with the project's Principal Investigator, who will serve as the QA Officer. In this project, the QA Officer's primary responsibilities are to conduct procedural and data reviews. Other project personnel are responsible for reporting quality problems to the QA Officer, who will then take corrective action.

B.3 Data Quality Parameters

Analytical data quality will be evaluated using the following data quality parameters:

- accuracy
- precision
- comparability
- completeness
- representativeness
- method detection limit

These data quality parameters will be used to determine if the data quality objectives of the demonstration have been met and are discussed in the subsections below.

B.3.1 Accuracy

Accuracy is the degree of agreement of a measurement with a "true" value. For the SERS method, accuracy will first be determined using matrix spike and laboratory control samples prepared under controlled conditions in the laboratory. Since the absolute concentrations are known, the accuracy of SERS can be assessed as percent recovery using the following formula (where perfect accuracy is indicated by 100% recovery):

$$\text{Percent Recovery} = 100([A]-[B])/[C] \quad (1)$$

where [A] = concentration of the analyte measured in a spiked sample
[B] = concentration of the analyte measured in an unspiked sample
[C] = concentration of analyte added to the spiked sample

Percent recoveries will be compared against HPLC limits set forth in Method 8000 as referenced in method 8330.

For split, well-mixed water samples analyzed soon after collection in the field, the major factor affecting accuracy is the subsequent analytical methodology, which is comprised of sample preparation and analysis steps.

In this project, field analytical accuracy will be evaluated by two methods using Method 8330 results as the "true" concentrations. First, accuracy estimates will be derived from linear regression analysis of the field method concentration estimates (y_i) versus Method 8330 results (x). For perfect accuracy, the least squares linear regression model would have a y-intercept of zero and a slope of 1.00. Deviations from that ideal can be statistically tested by computing confidence limits (often with $\alpha = 0.05$, but other levels can be chosen) around the slope (b_1) and intercept (b_0) in the model:

$$y = b_0 + b_1x \quad (2)$$

where y is the predicted value for the field method

The residual variance is given by:

$$\text{Residual Variance} = \text{sum}(y_i - y)^2 / (n - 2) \quad (3)$$

where n is the total number of field method estimates in the regression model

When the intercept confidence limits bracket zero and the slope confidence limits bracket 1.00, there is no significant bias in the field method (at the level chosen for the decision). Accuracy can then be expressed as the mean relative percent difference between field estimates and predicted concentrations.

Individual relative percent differences (RPD) are given by:

$$\text{RPD} = 100(y_i - y)/y \quad (4)$$

If the plotted data suggests curvature, the significance of the curvature can be tested by comparing Residual Variances of the linear and quadratic least squares models.

B.3.2 Precision

Precision is a measure of the closeness of agreement among replicate measurements made under defined conditions. Since the field measurements will be made in duplicate with separate aliquots carried through the entire analysis method, agreement of these duplicate results can be used to evaluate precision. One expression of precision is the relative percent difference (RPD') which is given by:

$$RPD' = 200([A] - [B])/([A] + [B]) \quad (5)$$

where [A], [B] are paired (duplicate) concentration estimates

The mean RPD' for the n pairs of measurements is one estimate of precision. In addition, if either the un-transformed or transformed paired differences form a normal distribution, a pooled estimate of the standard deviation (S_{yp}) can be computed from:

$$S_{yp} = \{\text{sum}([A]-[B])^2/2n\}^{1/2} \quad (6)$$

where n is the number of differences

B.3.3 Comparability

Comparability is a qualitative parameter that expresses the confidence that two data sets may be compared. The use of split samples that minimize variances outside the reference and field methods provides the necessary confidence that Method 8330 and field method data can be compared in this demonstration.

B.3.4 Completeness

Completeness of the demonstration will be assessed on the basis of percent valid data collected, which is calculated as follows:

$$\text{Completeness} = 100(\text{valid data}/\text{total data}) \quad (7)$$

The completeness goal for this project is 90%, with a minimum of 10 wells analyzed.

B.3.5 Representativeness

Representativeness is another qualitative parameter that expresses the degree to which sample data accurately and precisely represent a characteristic of a population, sampling point, or environmental condition. The use of split, paired samples minimizes reliance on representativeness as a necessary condition for quality assurance.

B.3.6 Method Detection Limit

The Method Detection Limit (MDL) describes the lowest concentration of an analyte that can be measured by an analytical method. MDLs will be determined using the following formula:

$$\text{MDL} = t_{(n-1)} * s \quad (8)$$

where s is the standard deviation of seven replicate analyses and $t_{(n-1)}$ is the student's t -value for a one-tailed test at the 99% confidence level with $(n-1)$ degrees of freedom

The Practical Quantitation Limit (PQL) is the lowest concentration that can be reliably achieved during routine operating conditions and is typically two to five times the calculated MDL.

B.4 Calibration Procedures, Quality Control Checks, and Corrective Action

This demonstration relies heavily on the use and comparison of results from three analytical methods, each with its own calibration procedures, quality control checks, and corrective actions. The sections below discuss these plan elements with regard to each analytical method.

B.4.1 U.S. EPA SW-846 Method 8330

Method 8330 is included as Appendix B to the Demonstration Plan and calibration procedures for the HPLC instrument are prescribed in Section 7.3 of that document. In accord with standard EPA SW-846 8000 series method guidance, initial instrument response and retention time calibration standards will be prepared and analyzed for each analyte of interest at five concentration levels bracketing the working range of the detector. The explosive standards will be either prepared from Standard Army Reference Material (SARM) or purchased as dilute solutions from Radian International. After the HPLC has been calibrated and a retention time window has been established for each analyte, three daily checks of instrument calibration are recommended and will be performed. QC procedures are described in Section 8 of Method 8330 and involve analysis of control samples and reagent blanks in accordance with good, standard analytical practice. Records of standards preparation, calibration results and maintenance, if any, required to correct instrument performance will be maintained in the project laboratory notebook discussed in the final two sections of this appendix

In the event that calibration checks fail, corrective action (i.e., maintenance and re-calibration) will be taken by the analyst to restore and re-quantify instrument performance. The criteria for calibration failure are one or more retention times outside the respective retention time windows, and/or a response that differs by more than $\pm 15\%$ of the value determined during initial instrument calibration. Calibration will be checked immediately after any instrument maintenance.

B.4.2 SERS

The Raman instrument will be calibrated for response to explosives as described in the previous section for Method 8330. The same analytical standards can be used and results will also be recorded in the laboratory notebook along with any changes in procedure, maintenance, or other corrective actions. Response calibration will be checked three times each day, and deviation by more than $\pm 20\%$ from the initial calibration value will be considered a calibration failure requiring corrective action (maintenance and/or re-calibration). Reagent blanks and controls will be tested with the same frequency as for Method 8330 above.

Wavelength calibration of the Raman instrument will be performed with a combination of a neon calibration lamp source and wavelength shift standard (naphthalene). Wavelength calibration will also be checked three times daily, and deviation in the peak position of any band by more than 5 cm^{-1} from the initial value will require that corrective action (i.e., maintenance and/or re-calibration) be taken.

Groundwater samples will be analyzed in duplicate, with 20% being the allowed difference before additional replicates must be analyzed. Data will be excluded from analysis only on a sound statistical basis.

B.4.3 Colorimetry

The colorimetric field screening procedures use a spectrophotometer for chemical analysis. QA/QC procedures for the colorimetric methods are outlined in references 2, 7, and 16 and generally follow the approaches described for Method 8330 and SERS in the previous sections of this QAPP. Response calibration will be performed as for Method 8330 and SERS, with calibration checks, controls, and blanks analyzed with the same frequency as well. Deviation of a response calibration "check standard" by more than $\pm 20\%$ from its original response will necessitate that corrective action (i.e., maintenance and/or re-calibration) be taken. Because the spectral features (bands) in spectrophotometry are extremely broad, careful calibration and regular monitoring of the wavelength stability of the spectrophotometer is not necessary as it is for the Raman spectrometer. As for SERS, water samples will be prepared and analyzed in duplicate with an allowable difference in the final results of 20% before more replicate analyses must be performed.

B.5 Demonstration Procedures

The demonstration procedures are presented in Section 3 of the Demonstration Plan.

B.6 Calculation of Data Quality Indicators

The calculation of data quality indicators is described in Sections C.3 and C.4.

B.7 Performance and System Audits

The ARA PI will have responsibility for overseeing the performance of ARA personnel and the CRREL PI will oversee the work of CRREL personnel. These managers are responsible for ensuring that procedures outlined in the Demonstration Plan and this QAPP are being followed. Data review will be conducted by the QA Officer identified in Section C.2 or his/her designee.

B.8 Quality Assurance Reports

Due to the short duration of the demonstration, separate quality assurance status or audit reports are not necessary. QC sample results and other quality related data will be included as part of regular technical reporting.

B.9 Data Format

Data will be collected with a computer or by manual recording in a laboratory notebook. The laboratory notebook will be maintained according to American Chemical Society guidelines, which ensure high quality recording of scientific data. The notebook will be used to record all non-electronic data, procedures used, quality checks, experimental observations, corrective actions and comments during the demonstration.

B.10 Data Storage and Archiving Procedures

SERS spectra will be recorded and stored electronically. HPLC chromatograms will be recorded and stored on integrator paper. All other data will be contained in the laboratory notebook. Backup copies of all electronic data will be made and stored on CD-ROM at the New England Division of ARA. The ARA Principal Investigator will maintain all data and other records related to the demonstration. In the event of a change of key personnel the data records will be retained in the division.

APPENDIX C

WATER SAMPLE RESULTS

ALAAP Groundwater Sample Results

Sample ID	Field				Lab							Ref Lab		
	TNT SERS	2,4-DNT SERS	TNT+DNT SERS	Color	TNT SERS	2,4-DNT SERS	TNT+DNT SERS	Color	TNT LC	2,4-DNT LC	TNT+DNT LC	TNT LC	2,4-DNT LC	TNT+DNT LC
GW-TA-01BR	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GW-TA-02BR	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GW-AF-02BR	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GW-AF-05BR	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GW-AF-06BR	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GW-AF-07BR	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GW-AF-08BR	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GW-AF-09BR	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GW-AF-11BR	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
GW-16-08BR	nd	nd	nd	Dye	nd	nd	nd	Dye	nd	nd	nd	nd	nd	nd
GW-20-04BR	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3	nd	nd
GW-18-001	201	57	258	220	185	62	247	261	196	71	267	141	37	178
GW-18-001-Top	178	24	202	209	153	29	182	184	163	31	194	nr	nr	nr
GW-18-001-Bottom	213	62	275	268	240	73	313	278	248	80	328	nr	nr	nr
GW-18-003	0	316	316	343	0	247	247	309	0	297	297	0	227	227
GW-18-003-Top	0	289	289	326	0	263	263	248	0	291	291	nr	nr	nr
GW-18-003-Bottom	0	193	193	214	0	177	177	170	0	210	210	nr	nr	nr
GW-18-08BR	0	27	27	19	0	25	25	17	0	22	22	0	14	14
GW-18-08BR-Top	0	17	17	16	0	19	19	16	0	16	16	nr	nr	nr
GW-18-08BR-Bottom	0	573	573	605	0	660	660	678	0	598	598	nr	nr	nr
GW-18-09BR	119	17	136	97	102	15	117	105	95	13	108	107	12	119
GW-18-09BR-Top	72	15	87	85	89	14	103	104	73	12	85	nr	nr	nr
GW-18-09BR-Bottom	11	5	16	12	12	4	16	15	14	6	20	nr	nr	nr
GW-BK-001	121	0	121	116	154	0	154	145	137	0	137	83	2	85
GW-AF-10BR	9	0	9	9	10	0	10	9	7	0	7	3	0	3
GW-17-20BR	25	31	56	51	28	33	61	45	29	29	58	21	22	43
GW-17-006	2374	2162	4536	4907	2578	2401	4979	4756	2865	2641	5506	3680	3300	6980
D-6	0	1523	1523	1734	0	1689	1689	1567	0	1786	1786	0	1668	1668
GW-04-05BR	0	4	4	Dye	0	5	5	Dye	0	4	4	0	2	2
GW-04-06BR	0	58	58	90	0	74	74	83	0	63	63	0	52	52
GW-04-001	0	7436	7436	7683	0	7919	7919	7253	0	8032	8032	0	7550	7550
GW-16-09BR	0	15	15	Dye	0	13	13	Dye	0	11	11	0	12	12

nd = not detected

nr = not run

Dye = dye interference

UMCD CPT Data - RDX							
Sample ID	In-situ SERS	Ex-situ SERS	Field Color*	Lab SERS	Lab LC	Lab Color	Ref Lab Color
DP-1	nd	12	13	10	8	11	15
DP-2	nd	6	8	9	7	6	5
DP-3	nd	nd	2	nd	nd	nd	nd
DP-4	nd	nd	1.5	nd	nd	nd	nd
DP-5	nd	nd	1.4	nd	nd	nd	nd
DP-6	nd	nd	1.8	nd	nd	nd	nd
Spike A (30 ug/L RDX)	28	34	32	35	26	37	29
Spike B (2.0 ug/L RDX)	nr	2.1	1.8	1.9	2.4	1.8	2.2

* Nitrate removal column not used resulting in false positive response for DP-3 to DP-6

nd = not detected

nr = not run

UMCD GAC Plant Data																						
	Field						Lab										Ref Lab Color		Ref Lab HPLC			
Sample ID	HMX SERS	RDX SERS	"RDX" Color	TNB SERS	TNT SERS	"TNT" Color	HMX SERS	RDX SERS	HMX LC	RDX LC	"RDX" Color	TNB SERS	TNT SERS	TNB LC	TNT LC	"TNT" Color	"RDX"	"TNT"	HMX	RDX	TNB	TNT
Influent	5	21	32	6	11	24	nd	28	4	33	26	8	13	6	12	20	21	22				
West	nd	12	16	nd	nd	nd	nd	9	nd	8	12	nd	nd	nd	nd	nd	10	nd				
East	nd	10	13	nd	nd	nd	nd	7	nd	5	8	nd	nd	nd	nd	nd	7	nd				
Effluent	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				
Spike (10 ug/L ea)	8	8	15	9	11	22	9	8	10	11	23	11	13	12	10	19	14	27				
Influent	nd	38	54	6	13	23	nd	47	3	37	49	6	9	4	12	14	61	19				
West	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				
East	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				
Effluent	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				
Influent	nd	20	22	6	8	11	nd	18	1	20	25	nd	11	5	9	13	37	15	3	23	4	7
West	nd	11	13	nd	nd	nd	nd	16	nd	15	16	nd	nd	nd	nd	nd	16	nd				
East	nd	12	15	nd	nd	nd	nd	13	nd	17	14	nd	nd	nd	nd	nd	15	nd				
Effluent	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				
Spike (10 ug/L ea)	10	11	18	12	10	19	9	9	11	10	23	9	11	11	11	22	31	24				

nd = not detected

"RDX" = nitramines

"TNT" = nitroaromatics

APPENDIX D

HEALTH & SAFETY PLAN

**HEALTH AND SAFETY PLAN
SUPPLEMENTAL REMEDIAL INVESTIGATION/FEASIBILITY STUDY
ALABAMA ARMY AMMUNITION PLANT - AREA B
CHILDERSBURG, ALABAMA**

FINAL

Submitted to:

**U.S. Army Environmental Center
SFIM-AEC-BCB
Aberdeen Proving Ground, Maryland 21010-5401**

Submitted by:

**Science Applications International Corporation
1710 Goodridge Drive
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